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REPORT NUMBER: 11111

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US ARMY MEDICAL INSTITUTE OF MEDICAL RESEARCH
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FORT SAM HOUSTON, TEXAS 78234

(1 October 1980 - 30 September 1981)

1 October 1981

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Prepared for:
US ARMY MEDICAL INSTITUTE OF MEDICAL RESEARCH
FOR DETRICK, FREDERICK, MD

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Basil A. Pruitt, Jr.

BASIL A. PRUITT, JR, MD, FACS
COLONEL, MC
COMMANDER & DIRECTOR

Accession For	
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DTIC TAB	<input type="checkbox"/>
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Justification	
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Distribution/	
Availability Codes	
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REPORT DOCUMENTATION PAGE		THIS REPORT IS REPORT DOCUMENTATION PAGE
1. REPORT NUMBER RCS MEDDH-288(R1)	2. REPORT NUMBER 1119768	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) US Army Institute of Surgical Research Annual Research Progress Report FY 1981		5. TYPE OF REPORT & PERIOD COVERED 1 Oct 80 - 30 Sep 81
7. AUTHOR(s) Basil A. Pruitt, Jr., COL, MC		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Institute of Surgical Research Fort Sam Houston, Texas 78234		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research & Development Command Fort Detrick Frederick, MD 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 3A161101A91C-00 3S161102BS10-00 3S162772A874-00
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE 1 October 1981
		13. NUMBER OF PAGES 14
		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		16a. DISSEMINATION/DOWNGRADING SCHEDULE
18. DISTRIBUTION STATEMENT (of this Report) Approved for public release. Distribution unlimited.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
19. SUPPLEMENTARY NOTES		
20. KEY WORDS (Continue on reverse side if necessary and identify by block number)		
21. ABSTRACT (Continue on reverse side if necessary and identify by block number) This report documents the clinical and laboratory activities of the US Army Institute of Surgical Research during the fiscal year 1981. These activities include patient care, clinical investigation and laboratory research in the areas of burn injury and general trauma. Special emphasis is placed on the clinical management of burned patients and on studies related to prevention and treatment of burned wound infection.		

FOREWORD

In politics and sports, it is said that winning isn't everything -- it is the only thing. In medicine in general and in medical research in particular, professional credibility isn't everything -- it is the only thing.

Professional credibility can only be achieved by a combination of investigator expertise, clinical and laboratory experience, and dedication. The US Army Medical Department and the US Army Medical Research & Development Command have both promoted and supported the professional credibility of this Institute by facilitating assignment of investigators and clinical staff possessing the necessary expertise, ensuring the necessary patient density, and providing the financial support essential to conduct state-of-the-art biomedical research. Such support recognizes the importance of professional credibility in terms of quality of patient care, effectiveness of teaching, and excellence of research which combine to enhance career opportunities and recruitment attractiveness for the Institute and the US Army Medical Research & Development Command.

The Burn Team is not simply a shorthand term for the many individuals involved in the care of the burn patient but an all-encompassing classification of the multidisciplinary staff of this Institute which ensures optimum patient care, timely identification of clinically relevant problems requiring study, and the rapid development of effective investigative activities.

These research results as well as the clinical outcome of the many critically ill patients cared for at the Institute clearly establish and verify the professional credibility of both the Institute's Burn Team as a whole and the individual members of the Team.

Basil A. Pruitt, Jr.

BASIL A. PRUITT, JR., MD, FACS
Colonel, MC
Commander and Director

The opinions expressed above are the private views of the author and are not to be construed as official or as reflecting the views of the US Army Medical Research & Development Command, the Department of the Army or the Department of Defense.

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	087	3A161101A91C	087
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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)336	
3. DATE PREV SUMMARY ^a	4. KIND OF SUMMARY ^a	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. ORIGIN INSTN ^a	9. SPECIFIC DATA - CONTRACTOR ACCESS ^a	
80 10 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
62772A		3S162772A874		AF		164	
STOG 80-7.2:5							
11. TITLE (Proceed with Security Classification Code) ^a							
(U) Clinical Operation, Center For Treatment of Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
50 07		Cont		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
Not Applicable				PRECEDING			
a. DATES/EFFECTIVE:				FISCAL YEAR		FUND (in thousands)	
b. NUMBER:				1981		50.0	
c. TYPE:				1982		50.0	
d. KIND OF AWARD:						1,156	
e. CUM. AMT.						1,300	
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
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22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATOR:			
				NAME: Basil A. Pruitt, Jr., MD, COL, MC			
				NAME:			
				POC: DA			
22. KEYWORDS (Proceed Each with Security Classification Code) (U) Heterograft; (U) Resuscitation; (U) Homograft; (U) Air Evacuation; (U) Thermal Injury; (U) Inhalation Injury; (U) Topical Therapy; (U) Autograft							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Proceed last of each with Security Classification Code.)							
<p>23. (U) The Clinical Division of the US Army Institute of Surgical Research continues its role as a major specialized clinical treatment center for thermally injured military personnel. Its main objectives are the investigation and modification of new diagnostic and therapeutic methods for optimum care of the burn patient as well as dissemination of the scientific advances to military and civilian medical treatment centers.</p> <p>24. (U) Thermally injured patients both from the Continental United States and throughout the world are evacuated to the US Army Institute of Surgical Research for intensive inpatient therapy. Carefully controlled evaluation of the efficacy of many treatment modalities is undertaken.</p> <p>25. (U) 8010 - 8109. Two hundred twenty five seriously burned patients were admitted and treated during 1980. Active clinical research activities include evaluation of laminar flow isolation to delay burn wound colonization; assessment of L-triiodothyronine therapy following thermal injury; studies of pulmonary function following crystalloid and colloid intravenous fluid resuscitation; investigation of the metabolic response to and nutritional support of acutely burned patients; investigation into the neurohumeral changes following severe injury; and, assessment of wound management techniques has provided information for the care of burned and injured man.</p>							

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF
BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 January 1980 - 31 December 1980

Investigators:

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Anton J. Jirka, MD, Colonel, MC
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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

**REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF
BURNED SOLDIERS**

**US Army Institute of Surgical Research, Brooke Army Medical
Center, Fort Sam Houston, Texas 78234**

Period covered in this report: 1 January - 31 December 1980

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Reports Control Symbol MEDDH-288(R1)

Two hundred and twenty-five patients were admitted to the Clinical Division of the United States Army Institute of Surgical Research during the calendar year of 1980. The emphases of Clinical Division activities over the past year have continued in the areas of excellence of patient care as well as research in the areas of host response to injury and improved methods of burn treatment. Education of health professionals has also remained a principal activity. Major areas of research included assessment of the metabolic and neuroendocrine responses to injury and resuscitation. This report summarizes the activities of the Clinical Division of the U.S. Army Institute of Surgical Research during calendar year 1980; catalogs the responses to treatment and complications which contributed to morbidity and mortality.

**Autograft
Heterograft
Homograft
Thermal Injury**

**Topical Therapy
Resuscitation
Air Evacuation
Inhalation Injury**

CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

The Clinical Division, U.S. Army Institute of Surgical Research admitted 225 soldiers and other authorized patients with thermal, chemical, or electric injuries during the 1980 calendar year. Burn Teams of this Institute conducted 76 aero-medical evacuation flights for 97 patients (43% of admissions) of which 73 flights were within the Continental United States for a total of 92 patients. The OCONUS flights were to Buenos Aires, Argentina for three patients and Puerto Rico and Panama for one patient each. Forty patients (18%) were admitted to this Burn Center from Emergency Medical Services in the immediate San Antonio area. Seventy-four of the 225 patients (32.8%) were admitted within 24 hours following injury and 146 patients (64.8%) were admitted within 48 hours of injury. The ages of admitted patients ranged from 7 months to 92 years with an average age of 29.8 years. The following statistics are based on 242 patient dispositions during the calendar year 1980. One hundred eighty-six (76.8%) of 242 dispositions were male patients and 56 (23.1%) were female. The average burn size of all dispositions was 35.7% total body surface burn with a 16.6% average full thickness injury. Fifty-three patients were in the pediatric age group (age 15 and under) and 57 patients were in the older age group (45 years of age or older). The average patient's hospital stay was 41.3 days when convalescent leave was included in the calculation and 38.4 days when convalescent leave was subtracted. In the 242 patients there were 16 electrically injured patients, one patient struck by lightning, five chemically injured patients, and the remaining 220 patients with either flame or scald burns. The cause of burn injury is delineated in Table 2 and the source of admissions is identified in Table 1.

EDUCATION

The professional staff of the Clinical Division, U.S. Army Institute of Surgical Research conducted numerous educational exercises for military and civilian professional and paraprofessional personnel during 1980. A total of 13 resident physicians were attached to the U.S. Army Institute of Surgical Research for periods of one to two months during 1980, including three from Brooke Army Medical Center, three from Fitzsimons Army Medical Center, two from Letterman Army Medical Center, two from Pensacola Naval Air Station, one from Wilford Hall Air Force Medical Center, one from the University of Texas Health Science Center at San Antonio and one from William Beaumont Hospital in Royal Oak, Michigan. Medical students rotating at the Institute

of Surgical Research for clinical clerkship experience included two health profession scholarship students, two students from the Uniformed Services University of the Health Sciences, one from State University of New York-Downstate, one from University of Oklahoma College of Medicine, two from Louisiana State University College of Medicine and one from Baylor College of Medicine. In addition, two physicians spent two weeks TDY for training during 1980. Physicians from foreign countries visited the Institute for periods of time ranging from one day to six months and included four from Great Britain, three from Sweden, three from North Yemen, three from India, three from Japan, two from Belgium, two from West Germany and one from each of the following countries, The Peoples Republic of China, Norway, Australia, Canada, Mexico, Yugoslavia, Denmark, Pakistan, Thailand, Egypt and Israel. In addition, fully trained physicians visited from the University of Vermont, University of Illinois, Syracuse, New York and Puerto Rico. The Physical Therapy Branch of the Clinical Division had 30 military and civilian trainees and the Occupational Therapy Branch had 57 trainees in calendar year 1980. Twelve scientific publications appeared in refereed medical journals and 150 scientific presentations were conducted for military and civilian medical audiences. Numerous scientific presentations were made at the Academy of Health Sciences and various military installations throughout the Continental United States to include support of Operation Red Flag of the Air Force. In addition, weekly professional staff conferences were conducted for and by the Institute personnel.

MORBIDITY AND MORTALITY

Sixty-six of 242 patients died during calendar year 1980 (Table 7) for an overall mortality of 27.3%. Autopsies were performed in 64% of these hospital deaths. The average total body surface burn injury of patients who died was 64.3% with an average third degree burn of 41.8% and an average age of 39 years. Twenty-six of the 66 patients who died (39%) had inhalation injury as a primary diagnosis or antecedent to pneumonia as a cause of death. Ten children (18.9% of dispositions) died with an average burn size of 57.9% total body surface and an average full thickness burn of 33.2%. The average age of those children who died was 4.9 years and seven of these 10 patients had autopsies. Unusual causes of death included one patient with Phencyclidine Hydrochloride (PCP) toxicity and one patient with Candida tropicalis pneumonia. Infection continues to be the most frequent complication of burn injury. Sixty patients had bacteria recovered from blood cultures; the single most common organism being Coagulase + Staphylococcus aureus in 30 patients followed

by Pseudomonas aeruginosa in 16 patients and Klebsiella species in 10 patients. Burn wound sepsis was diagnosed in 28 patients and suppurative thrombophlebitis in five patients. Candida species were recovered from the blood of eight patients and Aspergillus spp. were recovered from burn wound biopsies of 15 patients. Viral infections included Herpes simplex virus in three patients and chickenpox in one patient.

Again in 1980 no patient required celiotomy for upper gastrointestinal hemorrhage, however, one patient required closure of a perforated gastric ulcer and nine patients had clinical evidence of upper gastrointestinal hemorrhage which responded to non-operative therapy and one patient had a perforated gall bladder from acalculous cholecystitis.

Thirty-nine patients had acute renal failure and two of these 39 were treated with hemodialysis. Acute myocardial infarction was diagnosed in nine patients and acute bacterial endocarditis was seen in five patients. Pulmonary problems included 74 patients with inhalation injury, bronchopneumonia in 55 and hematogenous pneumonia in two patients. One hundred and two patients (42.1%) had associated injuries to include 74 patients with inhalation injury, CNS injury in seven, multiple lacerations in nine, eye injuries in 13 and fractures in six patients.

STATISTICAL RESUME DURING CALENDAR YEAR 1980

Two hundred twenty-five patients were admitted to the Institute of Surgical Research and there were 242 dispositions during the same period. Subsequent data are based on dispositions. There were 186 males and 56 females with an average age of 29.8 years ranging from seven months to 92 years of age. Fifty-three patients (22%) were less than 15 years old and 57 (23.5%) were over 45 years of age. The average total burn per cent was 35.7% with 16.6% average extent of full thickness burn. The average hospitalization of all patients, excluding convalescent leave for active duty military was 38.4 days. One hundred forty-six patients were admitted within 48 hours of injury.

During 1980, 1,554 operative procedures were performed on 198 patients for an average of 6.4 procedures per patient. Five hundred thirty-two anesthetics were given 150 patients (4.2 per patient). One hundred forty-four patients received a total of 466,090 cc of blood (3237cc/patient).

Table 1 identifies the source of admission of patients during calendar year 1980; table 2 summarizes burn etiology; table 3 lists the effect of age and extent of injury on survival; and, table 4 lists mortality rate associated with increments of 10% total body surface burn for years 1977 through 1980. Table 5 summarizes survival of patients with extensive burns from 1958 through 1980 and Table 6 compares mortality before and after the use of topical chemotherapy of the burn wound.

SUMMARY

The U.S. Army Institute of Surgical Research, Clinical Division admitted 225 acutely injured patients during calendar year 1980. No essential changes in patient management occurred except for an increasing use of wound excision to expedite closure of the burn wound.

PRESENTATIONS:

Pruitt BA Jr: Fluid Resuscitation of Injured Man. Grand Rounds Dept of Surg, Yale Univ Med Sch, New Haven, CT 11 Jan 80.

Pruitt BA Jr: Management of Burns in the Community Hospital. AMA Winter Scientific Mtg, San Antonio, TX 14 Jan 80.

McManus WF: Management of Burns in the Community Hospital -- Wound Management. AMA Winter Scientific Mtg, San Antonio, TX 14 Jan 80.

McManus WF: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 21 Jan 80.

The following presentations were made at the Seaton Medical Center Austin, TX 23 Jan 80:

McCandless SA: Recent Trends and Developments in Burn Trauma

Terry J: Nursing Care of the Burn Patient

Zitzka C: Nutrition for the Burn Patient

Pruitt BA Jr: Opportunistic Infections in Burn Patients. Grand Rounds Dept of Surg, Univ of Mississippi, Jackson, MS 24 Jan 80

Terry J: Burn Care. Physician's Assistant Program BAMC, Ft Sam Houston, TX 29 Jan 80.

Benitez H: Burn Assessment and Early Management. BAMC Interns ER/AMIC Clinic, BAMC Ft Sam Houston, TX 4 Feb 80.

Terry J: Emergency Burn Care. Judson High School Health Care classes. Converse, TX 4 Feb 80.

Walters MJ: Complications of Burns. Intensive Care Nurse Clinician Course students, BAMC Ft Sam Houston, TX 8 Feb 80.

McManus WF: Treatment of Burns. Residents and staff, Wilford Hall USAF Hospital, Lackland AFB, TX 8 Feb 80.

Goldfarb IW: Classification of Burns. Intensive Care Nurse Clinician Course students, BAMC Ft Sam Houston, TX 11 Feb 80.

Terry J: Emergency Burn Care and Overview of Burn Nursing Sch of Nursing Florida State Univ, Tallahassee FL 11 Feb 80.

Terry J: Burn Nursing. Intensive Care Nurse Clinician Course students, BAMC Ft Sam Houston, TX 12 Feb 80.

Terry J: Nursing Care of the Burn Patient. Intensive Care Nurse Clinician Course students, BAMC Ft Sam Houston, TX 13 Feb 80.

Allie JC: Physical Therapy Management of Burns. 91J students Academy of Health Sciences, Ft Sam Houston, TX 14 Feb 80.

Pruitt BA Jr: Fluid Therapy of the Severely Injured Patient. Grand Rounds, Dept of Surgery. State Univ of NY Medical Sch at Stony Brook, NY 14 Feb 80.

Walters MJ: Complications of Burns. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 19 Feb 80.

Terry J: Emergency Burn Care and Overview of Burn Nursing. Army Reserve Group. BAMC Fort Sam Houston, TX 19 Feb 80.

McCandless SA: Physical Therapy and the Burn Patient. Intensive Care Nurse Clinician Course students, BAMC Ft Sam Houston, TX 20 Feb 80.

Pruitt BA Jr: Infection and Sepsis in Burn Patients. Grand Rounds. Dept of Surgery Wayne State Univ Med Sch, Detroit, MI 23 Feb 80.

Terry J: Burn Nursing. Medina Memorial Sch of Nursing. Hondo, TX 25 Feb 80.

Pruitt BA Jr: Pulmonary Complications in Burns. Grand Rounds Dept of Surg, Vanderbilt Univ, Nashville, TN 25 Feb 80.

McManus WF: Air Evacuation of the Acutely Injured Patient. 55th Anl Mtg Texas Public Health Assn, San Antonio, TX 26 Feb 80.

Benitez H: Treatment of the Burn Patient. Physical Therapy students, Academy of Health Sciences, Ft Sam Houston, TX 29 Feb 80.

Terry J: Burn Nursing. Physical Therapy students. Academy of Health Sciences, Ft Sam Houston, TX 29 Feb 80.

Allie JC: Physical Therapy Management of Burns. 65B students, Academy of Health Sciences, Ft Sam Houston, TX 29 Feb 80.

Benitez H: Surgical Procedures on Burned Patients. Operating Room Technicians, Santa Rosa Hospital, San Antonio, TX 4 Mar 80.

McManus WF: Infection Control in Burn Units. Assn for Practitioners in Infection Control. Biloxi, MS 6 Mar 80.

Pruitt BA Jr: 1) Modern Trends in Burn Management; 2) Management of Infection in the Trauma Patient. Suncoast Trauma Seminar. Univ of South Florida Med Sch, Tampa, FL 13-14 Mar 80.

Walters MJ: Medical Red Flag 2. Travis AFB CA 18-20 Mar 80.

Goldfarb IW: Nutritional Support. Mercy Hospital, San Diego, CA 23-25 Mar 80.

The following presentations were made at the American Burn Association Anl mtg in San Antonio, TX 27-29 Mar 80:

Pruitt BA Jr: Diagnosis of Burn Wound Infection
Becker RA: Suppression of Free Thyroid Hormone Concentrations in the Clinical Unstable Burn Patient.
Walters MJ: Corneal Problems in Burned Patients
McManus WF: Central Nervous System Infections in Burn Patients
Goodwin CW: Lack of Effect of Colloid Oncotic Pressure on Pulmonary Extravascular Lung Water in Thermally Injured Patients
Aulick LH: Effects of Injury and Bacteremia on Hepatic Blood Flow and Glucose Metabolism of Burn Patients
McManus AT: Studies on the Mechanisms of In Vitro Resistance to Silver Sulfadiazine
Zitzka CA: Computer-Assisted Nutritional Assessment of the Thermally Injured Patient
McManus WF: Prevention of Infection by External Means
Pruitt BA Jr: Debugging the Patient. Moderator Plenary Session

Benitez, H: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 2 Apr 80.

Pruitt BA Jr: Systemic Effects of Thermal Injury. Distinguished Visiting Scientist Lecture. Albany Medical College, Albany, NY 9 Apr 80.

Pruitt BA Jr: Care of Combat Injured Burn Patients. Walter Reed Intern Lecture Series. Walter Reed Army Medical Center, Washington, D.C. 10 Apr 80.

Mansour EH: Burn Assessment and Early Management. BAMC Interns EM/AMIC Clinic BAMC Ft Sam Houston, TX 14 Apr 80.

McManus WF: Burn Wound Care. In Service Nursing Service ISR, Ft Sam Houston, TX 16 Apr 80.

Goldfarb IW: Total Parenteral Nutrition. Intensive Care Nurse Clinician Course students, BAMC Ft Sam Houston, TX 21 Apr 80.

The following presentations were made to the Clinical Pastoral Chaplain's Course, BAMC, Ft Sam Houston, Tx 23 Apr 80:

Goldfarb IW: Traumatic Injury
Truscott J: Critical Care Nursing with Burn Patients

Syby C: Emergency Burn Care and Overview of Burn Nursing. Nursing students, Ranger Junior College, at BAMC Ft Sam Houston, Tx 24 Apr 80.

Terry J: Nursing Care of the Thermally Injured. Midwest Recruiting Command. Nursing students Southeast Missouri State Univ, Cape Girardeau, MO 23 Apr 80; Univ of Missouri Columbia, Columbia, MO 25 Apr 80; Southwest Missouri State Univ at Springfield, MO 26 Apr 80.

Yurt RW: Acute Burn Care and Fluid Resuscitation. Physician's Assistants Continuing Education program. Womack Army Hospital, Fort Bragg, NC 24 Apr 80.

The following presentations were made to the USAF Nursing Service, Scott AFB, Mo 5-7 May 80:

Walters MJ: Complications of Burns
Terry J: Nursing Care of the Burn Patient
Lockwood L: Air Evacuation of the Burn Patients

The following presentations were made at the Combat Casualty Care Course, Camp Bullis, TX on 5-8 May 80:

Yurt RW: Initial Evaluation and Treatment of Combat Casualties

Walters MJ, Fanning RK, Mansour EH and Benitez H: Burn Management; Chest Trauma; Abdominal Trauma and Evaluation of Extremity Trauma

Pruitt BA Jr: 1) Early Care and Resuscitation of Burn Patients; 2) Colloids in Shock; 3) Care of the Burn Wound; 4) Septic Complications of Burn Patients; 5) Pulmonary Complications of Burn Injury. Anl Mtg of Soc of Critical Care Medicine, San Antonio, TX 12-13 May 80.

Pruitt BA Jr: 1) Recent Advances in Burn Care; 2) Triage, Transportation and Early Management of Burn Patients; 3) Resuscitation of Burn Patients. Am Burn Assoc Seminar on Burn Management, Baltimore, MD 16-17 May 80.

McManus WF: Management of Burns. Anl Mtg Texas Medical Association, Houston, TX 17 May 80.

Pruitt BA Jr: 1) Management of Burns in the Multiple Injured Patient; 2) Resuscitation and Early Hemodynamic Changes in the Burn Patient. U.S. Army Medical-Surgical Conference, Garmich, West Germany. 20-21 May 80.

Terry J: Emergency Burn Care and Overview of Burn Nursing. 159th USAH Reserve Unit, BAMC Ft Sam Houston, TX 22 May 80.

McManus WF: The Mission and Function of the Institute of Surgical Research, North San Antonio Optimist Club, San Antonio, TX 29 May 80.

Pruitt BA Jr: Metabolic and Nutritional Consequences of Injury. Visiting lecturer McGill Univ. Montreal Canada 30 May 80.

Terry J: Burn Nursing. Baptist Memorial Hospital School of Nursing, San Antonio, TX 2 Jun 80.

Pruitt BA Jr: Prophylactic Antibiotics in Surgery. Univ of Tex Medical Center, San Antonio, TX 11 Jun 80.

Pruitt BA Jr: 1) Characteristics of Inhalation Injury; 2) Resuscitation Fluid Regimens: Limitations and Indications: Anl Mtg Japan Society for Burn Injury. Sapporo, Japan 19-20 Jun 80.

Terry J: Burn Nursing. Brackenridge School of Nursing, Austin, TX 23 Jun 80.

Terry J: Emergency Burn Care and Overview of Burn Nursing. Nursing students, Henderson County Junior College, BAMC, Fort Sam Houston, TX 26 Jun 80.

Walters MJ: Burn Assessment and Management. BAMC Interns AMIC-ER, Fort Sam Houston, TX 23 Jun 80.

McManus WF: Treatment of Burns. Officers Basic Course. Academy of Health Sciences, Fort Sam Houston, TX 1 Jul 80.

Terry J: Emergency Burn Care and Overview of Burn Nursing. 337th Reserve Unit. BAMC Ft Sam Houston, TX 7 Jul 80.

Walters MJ: Emergency Management of Burns. Emergency Room Residents BAMC, Fort Sam Houston, TX 10 Jul 80.

Terry J: Emergency Burn Care and Evacuation. Division of Emergency Medicine, Texas Department of Health, Pan American Univ, Edinburg, TX 10 Jul 80.

Yurt RW: Emergency Management of Burns. Residents Internal Medicine Service, Fort Sam Houston, TX 12 Jul 80.

Terry J: Burn Care. Physician's Assistants Course. Academy of Health Sciences, Fort Sam Houston, TX 17 Jul 80.

Yurt RW: Burns. Officers Basic Course. Academy of Health Sciences, Fort Sam Houston, TX 23 Jul 80.

Walters MJ: Emergency Management of Burns. Physician's Assistants (Reservists). Academy of Health Sciences. Fort Sam Houston, TX 24 Jul 80.

Walters MJ: Classification of Burns. Intensive Care Unit Clinician Course students, BAMC, Fort Sam Houston, TX 25 Jul 80.

Walters MJ: Complications of Burns. Intensive Care Nurse Clinician Course students, BAMC Fort Sam Houston, TX 1 Aug 80.

Terry J: Nursing Care of the Thermally Injured. Intensive Care Nurse Clinician Course students, BAMC Fort Sam Houston, TX 4 Aug 80.

Terry J: The Burn Patient. Social Service Brooke Army Medical Center Fort Sam Houston, TX 6 Aug 80.

Terry J: Wound Care. Intensive Care Nurse Clinician Course students, BAMC Fort Sam Houston, TX 7 Aug 80.

Allie J: Physical Therapy Management of Burns. Intensive Care Nurse Clinician Course students, Fort Sam Houston, TX 11 Aug 80.

Mansour EH: Treatment of Burns. Officers Basic Course. Academy of Health Sciences, Fort Sam Houston, TX 13 Aug 80.

Becker R: Nutritional Support of the Critically Ill. Intensive Care Nurse Clinician Course students, BAMC Fort Sam Houston, TX 15 Aug 80.

Pruitt BA Jr: Current Treatment of the Extensively Burned Patient. Grand Rounds. Forsyth Hospital, Winston-Salem, NC 19 Aug 80.

Terry J: Emergency Burn Care and Evacuation. Dept of Emergency Medicine, Texas Dept of Health & A. Webb Roberts Center for Continuing Education, Austin, TX 21 Aug 80.

Terry J: Emergency Burn Care. Clinic BAFB, Brooks Air Force Base, TX 10 Sep 80.

Benitez H: Treatment of Burns. Officers Basic Course. Academy of Health Sciences, Fort Sam Houston, TX 17 Sep 80.

McManus WF: Burn Wound Infection. The American Association for the Surgery of Trauma Anl mtg. Phoenix, AZ 18 Sep 80.

McManus WF: Current Treatment of Thermal Injury. Musselman Surgical Symposium, University of Nebraska, Omaha, NE 19 Sep 80.

Henley MH: Pain and Suffering in Care of Burn Patients. Hastings Center, Hastings on the Hudson, NY 26 Sep 80.

Pruitt BA Jr: Stress Management in Burn Patient Care. Hastings Center. Hastings on the Hudson, NY 26 Sep 80.

Pruitt BA Jr: 1) Initial Evaluation and Treatment of Burns; 2) Diagnosis and Treatment of Common Complications of Burn Injury. American Medical Tennis Association, New Braunfels, TX 28 Sep 80.

Pruitt BA Jr: Recent Advances in Burn Care. Anl Claims Managers Mtg. Argonaut Insurance Co., San Antonio, TX 1 Oct 80.

Pruitt BA Jr: Fluid Resuscitation of Burn Patients. NIH Consensus Conf. Washington, D.C. 9 Oct 80.

Yurt RW: 1) Modern Burn Care; 2) Third Space Fluid Loss; 3) Nutrition and the Trauma Patient; and 4) Aeromedical Evacuation. Trauma Symposium Baptist Medical Center, Jacksonville, FL 9-11 Oct 80.

Allie JC: Physical Therapy and the Burn Patient. 91J students, Academy of Health Sciences, Fort Sam Houston, TX 14 Oct 80.

Pruitt BA Jr: Moderator. Surgical Forum Session. American College of Surgeons Anl Mtg. Atlanta, GA 22 Oct 80.

Benitez H: Treatment of Burns. Officers Basic Course. Academy of Health Sciences, Fort Sam Houston, TX 27 Oct 80.

McManus WF: Evacuation of the Burn Patient. 6th Cav of Fort Hood, TX, BAMC Fort Sam Houston, TX 29 Oct 80.

Walters MJ: Burn Management. Medical Red Flag III, Scott AFB, MO 28-30 Oct 80.

Maguire MS: Sports Medicine and the Role of the Physical Therapist. Physical Therapy students at UTSA HSC, San Antonio, TX 31 Oct 80.

The following presentations were made at Moorhead State University, Moorhead, MN 8 Nov 80:

Mansour EH: Burn Management

Terry J: Emergency Burn Care and Evacuation

Maguire MS: Physical Therapy for Burn Patients

Strieper GE: Burn Nursing. Association of Surgical Technologists, San Antonio, TX 15 Nov 80.

The following presentations were made at the Trauma and Disaster Symposium, Univ of Ohio, Columbus, OH 16 Nov 80:

Mansour EH: Burn Assessment and Early Management

Terry J: Nursing Care of the Burn Patient

Pruitt BA Jr: The Initial Evaluation and Treatment of Burns. Anl Mtg Southern Medical Association, San Antonio, TX 16 Nov 80.

Terry J: Nursing Care of Thermally Injured. Midwest Recruiting Command, Nurses at Univ of Wisconsin, Milwaukee 17 Nov 80; School of Nursing Elmhurst College, Elmhurst, IL 17 Nov 80; Westside VA Hospital, Chicago, IL 18 Nov 80; St. Xavier College School of Nursing, Chicago, IL 19 Nov 80; DePaul Univ School of Nursing, Chicago, IL 19 Nov 80; Marian College of Fond du Lac, Fond du Lac, WI 21 Nov 80.

Terry J: Burn Nursing. BAMC Operating Room Nurses Course, BAMC, Fort Sam Houston, TX 2 Dec 80.

Allie JC: Physical Therapy and the Burn Patient. Physical Therapists from the Air Force. ISR Fort Sam Houston, TX 10 Dec 80.

Pruitt BA Jr: 1) Renal Complications of Burn Injury; 2) Pulmonary Complications of Burn Injury; 3) Inhalation Injury; 4) Topical Antimicrobial Treatment of the Burn Wound; 5) Diagnosis and Treatment of Burn Wound Infection; 6) Unsolved Problems and Needs in Burn Care. International Society for Burn Injuries Seminar, Denver, CO 12 Dec 80.

PUBLICATIONS

1. Aulick LH, Baze WB, McLeod CG Jr et al: Control of blood flow in a large surface wound. *Ann Surg* 191:249-258, Feb 80.
2. Becker RA, Vaughan GM, Goodwin CW Jr et al: Plasma nor-epinephrine, epinephrine, and thyroid hormone interactions in severely burned patients. *Arch Surg* 115:439-443, Apr 80.
3. Pruitt BA Jr, McManus WF, Kim SH et al: Diagnosis and treatment of cannula-related intravenous sepsis in burn patients. *Ann Surg* 191:546-554, May 80.
4. Langlinais MS, Myers WD, Merrill RH: Scanning electron microscopic observations on glomeruli. *Arch Pathol Lab Med* 104:308-312, Jun 80.
5. Powanda MC, Villarreal Y, Rodriguez E et al: Redistribution of zinc within burned and burned infected rats. *Proc Soc for Exp Bio & Med* 163:296-301, 1980.
6. Treat RC, Sirinek KR, Levine BA et al: Air evacuation of thermally injured patients: Principles of treatment and results. *J Trauma* 20:275-279, Apr 80.
7. Levine BA, Sirinek KR and Pruitt BA Jr: Cimetidine prevents gastrointestinal edema associated with stress. *J Trauma* 20:464-467, Jun 80.
8. McManus AT, Moody EE and Mason AD Jr: Bacterial motility: A component in experimental *Pseudomonas aeruginosa* burn wound sepsis. *Burns* 6:235-239, Jun 80.
9. Wilmore DW, Goodwin CW, Aulick LH et al: Effect of injury and infection on visceral metabolism and circulation. *Ann Surg* 192:491-504, Oct 80.
10. Becker RA, Wilmore DW, Goodwin CW Jr: Free T_4 , Free T_3 and reverse T_3 in critically ill, thermally injured patients. *J Trauma* 20:713-721, Sep 80.
11. Mason AD Jr: The mathematics of resuscitation: 1980 presidential address, American Burn Association. *J Trauma* 20:1015-1020, Dec 80.
12. Powanda MC: Host metabolic alterations during inflammatory stress as related to nutritional status. *Amer J Veterinary Res* 41:1905-1911, Nov 80.

Table 1. Source of Admission, 1980

Area	A	AD	AF	AFD	N	ND	VAB	Other	TOTAL
1st Army	4	0	0	0	0	0	3	0	7
3rd Army	1	4	2	4	2	0	4	16	33
5th Army	11	18	3	7	4	2	21	86	152
6th Army	2	1	2	1	0	0	0	2	10
Germany	3	0	3	1	2	0	0	0	9
Korea	1	1	0	0	0	0	0	0	2
Turkey	0	0	1	0	0	0	0	0	1
Okinawa	0	0	0	1	0	0	0	0	1
Hawaii	0	0	0	0	3	1	1	2	7
Japan	0	0	0	0	14	0	0	0	14
Panama	1	0	0	0	0	0	0	0	1
Mexico	0	0	0	0	0	1	1	1	3
Argentina	0	0	0	0	0	0	0	3	3
Puerto Rico	0	0	0	0	0	0	0	1	1
	23	24	11	14	25	4	30	111	242

A - Army
 AF - Air Force
 D - Dependent
 Other: Civilian Emergency
 US Public Health Service Beneficiary
 Bureau of Employees Compensation Beneficiary
 N - Navy, Marine Corps & US Coast Guard
 VAB - Veterans Administration Beneficiary

Table 2. Burn Etiology, 1980 - 242 Dispositions

Causes	Number of Patients	% Disposition	Deaths	% Mortality
Gasoline, Diesel & Kerosene	45	18.6%	13	28.9%
Structural Fires	45	18.6%	19	42.2%
Motor Vehicle Accidents	8	3.3%	2	25.0%
Aircraft Accidents	9	3.7%	1	11.1%
Open Flames	23	9.5%	7	30.4%
Electrical	16	6.6%	1	6.3%
Hot Liquids	42	17.4%	6	14.3%
Chemical	6	2.5%	1	16.7%
Butane, Propane or Natural, Sewer Gas Exp.	24	9.9%	10	41.7%
Welding	1	0.4%	1	100.0%
Smoking Clothes Ignited	6	2.5%	3	50.0%
Bomb, Shell, Simulator Grenade, Gunpowder Exp.	4	1.7%	0	0.0%
Others	10	4.1%	2	20.0%
Contact	3	1.2%	0	0.0%
TOTAL	242		66	

Table 3. Age, Body Surface Involvement & Mortality, 1980

Age (Yrs)	Per Cent Burn										Total		% Mortality
	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Cases	Deaths	
0-1	1	1	0	2(1)	1(1)	0	0	0	0	0	5	2	40.0
1-2	3	3	3	1	1(1)	2(1)	0	0	0	0	13	2	15.4
2-3	1	1	2	0	0	0	0	0	0	0	4	0	0.0
3-4	0	2	2	0	0	0	1(1)	0	0	0	5	1	20.0
4-5	0	2	0	2	0	0	0	0	0	0	4	0	0.0
5-10	0	4	1	2	0	1(1)	1(1)	1(1)	0	0	10	3	30.0
10-15	3	2	3	2	0	0	1(1)	1(1)	0	0	12	2	16.7
15-20	8	6	2	3	1	1	2(1)	2(2)	2(1)	0	27	4	14.8
20-30	5	5	11	10	6(1)	5(1)	1	2(2)	3(2)	2(2)	50	8	16.0
30-40	4	5	5	5	2	5(2)	4(4)	1(1)	4(4)	2(2)	37	13	35.1
40-50	3	4	5	5	3(1)	4(3)	1	2(2)	0	1(1)	28	7	25.0
50-60	5	3(1)	4(1)	2	1(1)	3(2)	2(2)	1(1)	3(3)	0	24	11	45.8
60-70	1	4	3(1)	1	2(2)	2(1)	2(1)	1(1)	0	1(1)	17	7	41.2
70-80	0	0	0	1(1)	1(1)	1(1)	0	0	0	0	3	3	100.0
80-90	0	0	0	1(1)	0	0	0	0	1(1)	0	2	2	100.0
90-100	0	0	0	0	0	0	1(1)	0	0	0	1	1	100.0

Total 34 42 41 37 18 24 16 11 13 6 242

Deaths 0 1 2 3 8 12 12 11 11 6 66

% Mortality 0 2.4 4.9 8.1 44.4 50 75 100 84.6 100 27.3

Table 4. Per Cent Body Surface Involvement and Mortality, 1977 - 1980

% Burn	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
(1977)											
No. Burned	37	35	32	46	24	20	18	12	6	4	234
Deaths	0	1	5	10	9	11	14	11	5	4	70
% Mortality	0	2.9	15.6	21.7	37.5	55	77.8	91.7	83.3	100	29.9
(1978)											
No. Burned	48	49	46	37	27	17	20	12	6	6	268
Deaths	0	4	6	10	9	8	12	12	2	6	69
% Mortality	0	8.2	13.0	27.0	33.3	47.0	60.0	100	33.3	100	25.8
(1979)											
No. Burned	50	49	35	23	32	29	16	10	15	8	267
Deaths	0	2	0	8	8	11	12	10	15	8	74
% Mortality	0	4.0	0	34.8	25.9	37.9	75.0	100	100	100	27.7
(1980)											
No. Burned	34	42	41	37	18	24	16	11	13	6	242
Deaths	0	1	2	3	8	12	12	11	11	6	66
% Mortality	0	2.4	4.9	8.19	44.4	50	75	100	84.6	100	27.3

Table 5. Survival and Death by Year for Patients
With Extensive Burns, 1958-1980

Year	Survivors (burns over 30%)			Deaths		
	No. Cases	Average % Burn		No. Cases	Average % Burn	
		Total	3 rd		Total	3 rd
1958	15	42.3	21.6	23	56.5	35.3
1959	29	43.1	20.6	24	63.1	38.1
1960	17	44.2	20.1	30	57.8	37.3
1961	18	44.2	25.0	31	58.0	39.7
1962	18	42.7	21.4	54	59.1	46.2
1963	28	45.8	19.6	57	69.0	41.0
1964	40	41.8	14.8	37	65.0	42.4
1965	47	43.8	21.0	33	66.0	33.4
1966	68	41.5	14.9	59	59.9	31.3
1967	103	42.7	13.3	51	59.9	32.3
1968	143	44.2	12.6	38	54.6	24.6
1969	113	43.2	11.1	70	58.7	26.4
1970	92	39.4	10.7	70	51.9	32.6
1971	63	41.9	14.0	68	60.8	38.0
1972	62	42.0	17.2	103	56.7	35.9
1973	47	43.7	19.6	113	60.3	36.2
1974	55	43.9	12.2	97	60.8	35.9
1975	80	46.1	14.7	94	61.3	32.8
1976	69	45.5	15.0	79	64.2	31.1
1977	66	42.2	14.4	70	56.9	29.0
1978	67	45.7	14.8	69	55.2	33.0
1979	61	45.4	13.4	74	65.0	37.0
1980	62	42.7	15.1	66	64.3	41.8

Table 6. Comparison of Burn Mortality Rates, 1962-1963 and 1964-1980

Years	Per Cent Burn														
	0-30			30-40			40-50			50-60			60-100		
	No.	%	Mortality	No.	%	Mortality	No.	%	Mortality	No.	%	Mortality	No.	%	Mortality
	Pts.	Deaths	Mortality	Pts.	Deaths	Mortality	Pts.	Deaths	Mortality	Pts.	Deaths	Mortality	Pts.	Deaths	Mortality
1962-63	140	6	4.3	36	16	44.4	36	22	61.1	23	18	78.3	55	49	89.1
1964-80	2302	76	3.3	648	126	18.6	555	178	32.0	392	192	49.0	729	619	84.9

Table 7. Cause of Death, 1980

Patient	Age	Sex	% Burn Total	PBD 30 Death	Cause of Death	
1	21	M	97	65	19	97% total body surface burn and extensive bilateral pneumonia, <i>Candida</i> species
2	30	M	94.5	90.5	11	94.5% total body surface burn with septic pancarditis and myocardial abscesses of the heart, organism <i>Staphylococcus aureus</i>
3	44	M	93	93	10	93% total body surface burn, inhalation injury with subsequent <i>Proteus mirabilis</i> pneumonia and septicemia
4	23	M	92	92	4	*92% total body surface burn with <i>Klebsiella</i> pneumonia septicemia and severe leukopenia secondary to silver sulfadiazine on admission
5	61	F	92	16.5	5	92% total body surface burn, inhalation injury, <i>Klebsiella</i> pneumonia and septicemia
6	35	M	91	79	19	*91% total body surface burn, severe inhalation injury bilaterally with subsequent bilateral pneumonia from <i>Staphylococcus</i> and enterobacter species with septicemia
7	50	M	87	77	4	*87% total body surface burn and enterobacter cloacae septicemia
8	32	F	85	79.5	85	*85% total body surface burn with <i>Staphylococcus aureus</i> and <i>Klebsiella</i> pneumonia with septicemia
9	56	M	85	76	6	<i>Staphylococcus aureus</i> pneumonia and septicemia
10	31	M	84.5	49.5	19	84.5% total body surface burn with <i>Pseudomonas aeruginosa</i> and <i>Providencia stuartii</i> pneumonia and septicemia
11	23	M	83	67.5	59	*83% total body surface burn, invasive burn wound sepsis <i>Pseudomonas aeruginosa</i> , and hematogenous pneumonia
12	38	M	82	55	15	82% total body surface burn, <i>Pseudomonas</i> pneumonia and <i>Pseudomonas</i> burn wound sepsis
13	86	M	81	51.5	7	*81% total body surface burn and probable acute myocardial infarction
14	16	F	81	50	3	81% total body surface burn, <i>Staphylococcal</i> pneumonia, suppurative thrombophlebitis and septicemia

* Autopsy not performed

Table 7. Cause of Death, 1980

Patient	Age	Sex	% Burn Total	30 Death	PBD Death	Cause of Death
15	55	F	81	25.5	6	81% total body surface burn, bilateral pneumonia and multiple pulmonary emboli
16	38	M	80.5	47	26	80.5% total body surface burn and acute myocarditis with micro abscesses, Staphylococcus aureus and burn wound invasion Pseudomonas aeruginosa
17	25	M	80	80	2	80% total body surface burn with inhalation injury
18	42	M	79	56.5	17	*79% total body surface burn with severe bilateral inhalation injury and subsequent Staphylococcal aureus pneumonia and septicemia
19	19	M	77.75	71.25	14	77.75% total body surface burn with invasive pseudomonas burn wound infection and septicemia
20	43	M	26	72	15	76% total body surface burn, severe inhalation injury with Staphylococcal pneumonia and septicemia
21	27	M	76	51.5	7	76% total body surface burn, acute myocardial infarction, Staphylococcus aureus pneumonia and septicemia
22	25	M	76	27.5	27	76% total body surface burn, acute inhalation injury with Pseudomonas aeruginosa pneumonitis and septicemia
23	13	F	73.5	64	8	73.5% total body surface burn and Pseudomonas aeruginosa burn wound sepsis
24	37	M	73	64	20	*73% total body surface burn, bilateral inhalation injury, bilateral pneumonia coagulase positive Staphylococcus aureus with septicemia
25	66	M	72	58	1	*72% total body surface burns with bilateral severe inhalation injury and subsequent bronchopneumonia
26	6	M	70.5	1	13	75% total body surface burn, acute myocardial infarction and Candida tropicalis pneumonia bilaterally
27	19	M	70	18	27	70% total body surface burn, invasive burn wound sepsis, severe inhalation injury and Staphylococcus aureus pneumonia and septicemia

* Autopsy not performed

Table 7. Cause of Death, 1980

Patient	Age	Sex	% Burn Total	3°	PBD Death	Cause of Death
28	54	F	70	3	13	70% total body surface burn with acute myocardopathy
29	56	M	69	11.5	19	69% total body surface burn with inhalation injury and complete occlusion of the left internal carotid artery with brain death
30	31	M	68.5	52	3	68.5% total body surface burn, acute myocardial degeneration and cerebral edema
31	39	M	68.5	50.5	2	*68.5% total body surface chemical burn with presumed pentachlorophenol (PCP) toxicity
32	5	F	68	51	1	68% total body surface burn with bilateral severe inhalation injury
33	3	M	65.5	44	5	65.5% total body surface burn and acute bronchopneumonia
34	92	M	65.5	29	4	65.5% total body surface burn and acute myocardial infarction
35	37	M	65	65	37	65% total body surface burn, gangrenous acalculous cholecystitis with peritonitis subhepatic abscess and septicemia
36	12	F	63.5	39.5	3	63.5% total body surface burn severe bilateral inhalation injury
37	63	M	62	57.5	18	62% total body surface burn bilateral Pseudomonas pneumonia with microabscess and Pseudomonas Invasive burn wound sepsis
38	17	M	60	42	8	60% total body surface burn, severe inhalation injury with Pseudomonas pneumonia bilateral and Pseudomonas septicemia
39	57	M	60	38	13	*60% total body surface burn, severe bilateral Staphylococcal aureus bronchopneumonia with septicemia
40	31	M	60	4.5	0	*60% total body surface burn with bilateral severe inhalation injury and acute myocardial infarction
41	40	M	59	28	1	Inhalation injury severe bilateral, septic endocarditis and acute myocardial infarction
42	44	M	58.5	22.5	22	Severe pneumonia bilaterally with septicemia

Autopsy not performed

Table 7. Cause of Death, 1980

Patient	Age	Sex	% Burn Total	% Death	PBD	Cause of Death
43	50	F	58	58	41	Invasive burn wound sepsis
44	38	F	58	45.5	31	Acute myocarditis and hemopericardium along with bilateral Pseudomonas bronchopneumonia
45	6	M	56.5	33	4	Pulmonary edema and pneumonia
46	52	M	55.5	15	13	*Bilateral pneumonitis organism Staphylococcus aureus
47	27	M	54	48	46	Severe inhalation injury complicated by bilateral pneumonitis organisms Providencia stuartii and Enterobacter aerogenes with septicemia
48	33	M	53.5	31	9	Inhalation injury, acute bronchopneumonia and multiple pulmonary emboli
49	40	M	53	51	16	Bilateral pneumonia organism Pseudomonas aeruginosa and Klebsiella species
50	15/12	F	53	15	10	Severe Staphylococcus aureus pneumonitis and septicemia
51	77	F	52.5	19	9	*Severe inhalation injury complicated by bilateral pneumonia organism Staphylococcus aureus with septicemia and acute myocardial infarction
52	68	F	51	20.5	6	*Acute inhalation injury with severe bilateral pneumonitis
53	77/12	M	48	41	25	*Burn wound sepsis Aspergillus species
54	24	M	46	20.5	6	*Severe inhalation injury, acute renal failure with massive necrosis and myoglobinuric nephropathy
55	55	M	44.5	19.5	21	Severe inhalation injury, pan-lobe pneumonia and lung abscesses secondary to the inhalation injury and septicemia
56	48	M	44	21.5	58	Bilateral bronchopneumonia mixed organisms Staphylococcus aureus and Pseudomonas aeruginosa with septicemia with the same organisms

Autopsy not performed

Table 7. Cause of Death, 1980

Patient	Age	Sex	% Burn Total	30 Death	PBD Death	Cause of Death
57	1	F	43	43	2	*Cerebral edema with cardio-respiratory arrest
58	67	F	41.5	11.5	77	*Severe inhalation injury with Pseudomonas pneumonia and septicemia
59	69	M	40	36.5	35	*Severe inhalation injury and bilateral pneumonitis with Pseudomonas aeruginosa
60	79	M	40	17.5	8	*Arteriosclerotic heart disease
61	84	M	39	39	50	Bilateral severe pneumonitis subsequent to inhalation injury
62	7/12	M	37.5	0	10	*Gram negative septicemia presumed source burn wound
63	70	F	32	15	85	Acute myocardial infarction and arteriosclerotic peripheral vascular disease with cerebral infarction from occlusion of right middle cerebral artery
64	63	F	27.5	24.5	88	*Inhalation injury with subsequent bilateral pneumonitis with Pseudomonas aeruginosa and Pseudomonas septicemia
65	50	M	26	17	70	Bilateral pneumonitis and lung abscesses with Pseudomonas aeruginosa and Pseudomonas septicemia
66	50	M	12.5	3	10	Massive pulmonary embolus

* Autopsy not performed

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

**REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF
BURNED SOLDIERS--ANESTHESIOLOGY**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 January 1980 - 31 December 1980

Investigator:

Anton J. Jirka, MD, MPH, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF
BURNED SOLDIERS--ANESTHESIOLOGY

US Army Institute of Surgical Research, Brooke Army Medical
Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 January 1980 - 31
December 1980

Investigator: Anton J. Jirka, MD, MPH, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

In the period covered in this report, 531 anesthetics were administered to 148 patients, an average of 3.59 anesthetics per patient. This is the second largest number of anesthetics administered at ISR since 1972. The most commonly used anesthetic agent was Ethraneⁿ (47.46%), followed by ketamine (34.46%), and nitrous oxide (13.37%). Due to the nature and combinations of procedures now performed, regional anesthesia is seldom used. An automatic oscillographic blood pressure monitor is presently used on all patients.

Anesthesia.

ANESTHESIOLOGY

PREOPERATIVE EVALUATION

Most burn patients are several days postinjury when first seen by the anesthesiologist. In the immediate postburn period, the time is used to gain abundant physiologic data from routine monitoring of various indices: hematologic (hematocrit, electrolytes, liver and renal function tests), pulmonary (arterial blood gases, respiratory rate, daily chest roentgenograms), cardiovascular (blood pressure, central venous pressure, cardiac index measured by use of Swan-Ganz catheters), and renal (urine output, urine chemistry), in addition to the usual preoperative patient interview and physical examination.

All patients, regardless of age, who have electrical injuries have a preoperative electrocardiogram performed to rule out possible myocardial damage.

PREOPERATIVE PREPARATION

All patients are kept NPO after 2400 the day prior to surgery with the exception of children, who may receive clear liquids up to five hours prior to surgery.

Due to extraordinary fluid requirements in most burned patients, an intravenous infusion, if not already in place, is begun the evening prior to surgery.

PREMEDICATION

Glycopyrrolate (Robinul^R) 0.005 mg/kg to a maximum dose of .4 mg, is given intramuscularly as premedication 30 minutes prior to anesthesia. Narcotic premedication is no longer routinely used.

FLUIDS

All fluids except hyperalimentation solutions are changed to D₅RL or RL on arrival in the operating room. Hyperalimentation solutions are continued throughout operative procedures.

TYPES OF ANESTHESIA

The pattern of anesthetic administration has changed from previous years and involves a greater use of enflurane and

ketamine and a lesser use of halothane and regional anesthesia. The reasons for this change will be discussed under individual agent headings. (Table 1)

TABLE 1. PRIMARY AGENTS

<u>AGENT</u>	<u>1979</u>		<u>1980</u>	
	<u>NUMBER</u>	<u>%</u>	<u>NUMBER</u>	<u>%</u>
ENFLURANE	324	58.59	252	47.46
KETAMINE	143	25.86	183	34.46
HALOTHANE	18	3.25	10	1.88
N ₂ O	38	6.87	71	13.37
LOCAL	29	5.24	15	2.82
OTHER	1	0.18	0	0

1. Enflurane (Ethrane^R)

Enflurane is a halogenated ether which has been commercially available for approximately the past seven years. It has a rapid induction with good muscle relaxation. Biotransformation amounts to less than 2% of an inhaled dose, a fact which perhaps accounts for the few clinical toxic effects observed in spite of the fact that increased plasma fluoride ion concentrations have been observed after administration to patients taking hepatic enzyme inducing drugs. Plasma fluoride levels in hypermetabolic burn patients during and after Ethrane administration have been measured and found not to be in the toxic range. Enflurane is presently the most commonly used anesthetic agent at the USAISR.

2. Halothane^R (Fluothane)

The use of halothane is avoided mostly for less than rational reasons related to descriptions of probable hepatotoxicity (incidence 0.7 per 1000) in the literature. Previous studies at the Institute of Surgical Research show its repeated use to be safe in the thermally injured patient, and the National Halothane Study showed halothane to be the anesthetic with the best overall mortality rate. It is a smooth anesthetic, unsurpassed as an agent for pediatric patients. This anesthetic is mainly used now for asthmatics, patients with digitalis toxicity, and children. Its use has decreased as to favor ketamine in the young age group.

3. Nitrous Oxide

This agent is used in concentrations of 50% or 60% with oxygen. It is used mainly in conjunction with other analgesic or anesthetic agents. Succinylcholine has not been used for any purpose in this unit for more than six years.

4. Ketamine

This agent is used both IM and IV to produce its characteristic dissociative state, with preservation of basal functions (breathing) and laryngeal reflexes plus stimulation of the cardiovascular system.

Unfortunately, ketamine shares with its parent compound, phencyclidine, the production of a high incidence of unpleasant hallucinogenic side effects. There seems to have been a "batch" difference in ketamine, and that possessed by ISR in the past had an almost 100% incidence of these effects. New methods of administering the drug, as well as various methods of premedication and patient preparation, appear to have reduced the unpleasant emergence reactions to a level where they are of little consideration in the well selected patient. Laryngospasm, airway obstruction and regurgitation can occur with ketamine. Pronounced blepharospasm prevents its use in eye cases. All ketamine anesthetics, other than in children, are preceded by IV droperidol (0.15 mg/kg) or diazepam (0.15-0.2 mg/kg).

5. Subanesthetic Ketamine

Subanesthetic ketamine (single dose 1.5-2 mg/kg IM) has not been used during this reporting period except for dressing changes where it is the anesthetic of choice. Tolerance to ketamine has been noted in several patients after repeated (greater than five) ketamine anesthetics. Ketamine is no longer used for Hubbard tank procedures. Although of limited value, sedation and narcotic analgesia, administered under direction of the surgical staff, have replaced ketamine for this use.

6. Regional Anesthesia

Regional anesthesia is generally considered one of the safest methods available, but its use in the thermally injured patient is limited for several reasons: sepsis and infection of the skin over or near the site of injection are contraindications for use, and multiple-site operations also limit the practicality of this method. Axillary block is the most common regional technique used at USAISR. However the tendency toward multiple procedures has decreased the usefulness of this technique.

MONITORING TECHNIQUES

A. CIRCULATION

1. Precordial and/or esophageal stethoscope
2. Peripheral pulse
3. Blood pressure. Direct arterial lines have been used when necessary. The Dinamap^R blood pressure instrument is routinely used for intraoperative blood pressure monitoring. Since it can be used over dressings and is noninvasive, it is a most practical method of monitoring blood pressure in our patient population.
4. CVP
5. Swan Ganz catheter
6. ECG
7. Sponge weight - rarely used
8. Urine output

B. RESPIRATION

1. Rate
2. Auscultation
3. Arterial blood gases

C. TEMPERATURE

In most cases a temperature monitor is employed. Because of the greatly increased evaporative heat losses in burn patients, hypothermia is a serious problem. Several methods are employed to maintain body temperature during anesthesia:

1. Ambient temperature is maintained at 82-87°F. This is probably the most important method to reduce heat loss.

2. The anesthetic gases may be heated and humidified.

3. A circle system which allows partial rebreathing of warm expired gases may be used to minimize heat loss. A Bain Circuite which achieves the same purpose is used in children.

4. Radiant heat lamps

5. The K-thermia heating blanket can also be used. It is probably used most effectively on children weighing less than 10 kg and for cooling febrile patients.

COMPLICATIONS

There was one intraoperative death during 1980. It occurred during arteriography in a 24 year old male. An autopsy was refused and the cause of death was not determined. This is the first intraoperative death at ISR since 1978.

PATIENT DATA AND OPERATIVE PROCEDURES

The following two tables illustrate overall anesthetic patient data for the years 1970 through 1980 (Table 2) and recent trends in operative procedures (Table 3).

TABLE 2. OVERALL PATIENT DATA, USAISR (1970-1980)

Year	No. of Patients	No. Patients Anesthetized (ISR Only)	Average Number Patients Anesthetized	Total Anesthetics Given at ISR	Average Anesthetics Per Patients Anesthetized
1970	321	198	61.7	497	2.51
1971	301	179	59.5	475	2.65
1972	301	183	60.8	575	3.14
1973	273	141	51.6	377	2.67
1974	226	123	54.4	380	3.09
1975	254	142	55.9	490	3.45
1976	277	139	50.2	476	3.43
1977	242	129	53.3	344	2.67
1978	268	151	56.3	435	2.88
1979	267	161	60.3	554	3.44
1980	243	148	60.91	531	3.59

TABLE 3. NATURE OF SURGERY, USAISR

PROCEDURE	1978		1979		1980	
	NUMBER PROCEDURES	%	NUMBER PROCEDURES	%	NUMBER PROCEDURES	%
EXCISION	90	19.3	212	30.15	269	37.36
AUTOGRAFT	269	59.9	372	52.91	318	44.17
ORTHOPEDIC	33	7.1	34	4.84	38	5.28
CHONDRECTOMY	4	0.9	1	0.14	4	0.56
EYE AND LID	6	1.3	21	2.99	17	2.36
INTRA-ABDOMINAL	6	1.3	8	1.13	1	0.14
PLASTIC	6	1.3	3	0.43	5	0.69
OTHER	50	10.8	52	7.39	68	9.44
TOTAL	464	100%	703	100%	720	100%

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT ORIGIN: SYMBOL	
				DA OG 6975	81 10 01	DD-DRSB(AF)0396	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DRG/N INSTN	9. SPECIFIC DATA- CONTRACTOR ACCESS	10. LEVEL OF EFF
80 10 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
11. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
12. PRIMARY	62772A	3S162772A874		AF	161		
13. XXXXXXXXXX							
14. XXXXXXXXXX	STOG 80 -7.2:5						
15. TITLE (Proceed with Security Classification Code)							
(U) The Cardiopulmonary Response to Thermal Injury in Burned Soldiers							
16. SCIENTIFIC AND TECHNOLOGICAL AREA							
003500 Clinical Medicine							
17. START DATE		18. ESTIMATED COMPLETION DATE		19. FUNDING AGENCY		20. PERFORMANCE METHOD	
76 10		Cont		DA		C. In-House	
21. CONTRACT/GRANT				22. RESOURCES ESTIMATE		23. PROFESSIONAL MAN YRS	
Not Applicable				PREVIOUS		1.0	
24. DATES/EFFECTIVE:				FISCAL YEAR		25. FUNDS (in thousands)	
EXPIRATION:				1981		50	
26. NUMBER:				CURRENT		100	
27. TYPE:				1982		2.0	
28. KIND OF AWARD:				F. CUM. AMT.			
29. RESPONSIBLE S&T ORGANIZATION				30. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr., MD, COL, MC				NAME: Cleon W. Goodwin, Jr., M.D.			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2968			
31. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
32. TECHNICAL OBJECTIVE, 33. APPROACH, 34. PROGRESS (Furnish individual paragraphs identified by number. Proceed rest of each with Security Classification Code.)							
<p>23. (U) To evaluate systemic and cardiopulmonary changes in burned soldiers and the influence of fluid resuscitation. To study by both invasive and noninvasive techniques pulmonary and myocardial function in burned and burned-infected patients.</p> <p>24. (U) Hemodynamic flow and pressure changes and ventilation sensitivity are studied in burn patients during and after resuscitation. Cardiac output and lung water are studied by a standardized rebreathing indicator-dilution technique and by an intravascular double indicator dilution method. Comparisons between these two methods are made.</p> <p>25. (U) 8010 - 8109. Cardiac index and lung water were determined in five thermally injured patients (mean age 21 years; mean burn size 55% TBS) in the first seven postburn days by a rebreathing method utilizing two differentially diffusible gases (RBLW) and the thermal-green dye double indicator-dilution technique (TGLW). Catheters were placed in the pulmonary artery (PA) and femoral artery (FA), and the mean transit times for the green dye and the thermal indicators were computed. RBLW, determined by a time and blood flow independent method, increased significantly over the study (+60% p < .05). TGLW, measured by the bolus injection thermal dye method, slightly decreased as blood flow rose. Transport functions, calculated from the input (PA) and</p>							

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(ARM&S)	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. ORIGIN INSTN ^a	9. SPECIFIC DATA ^a CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
80 10 01	D. CHANGE	U	U	NA	NL		
10. NO./CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
A. PRIMARY		62772A		3S162772A874			
B. MINOR							
C. MINOR		STOG 80 - 7.2:5					
11. TITLE (Precede with Security Classification Code) ^a							
(U) The Cardiopulmonary Response to Thermal Injury in Burned Soldiers							
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003500 Clinical Medicine							
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76 10		Cont		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
Not Applicable				FISCAL YEAR		20. FUNDS (in thousands)	
A. DATES/EFFECTIVE:				PREVIOUS		1.0	
B. NUMBER:				1981		50	
C. TYPE:				CURRENT		2.0	
D. KIND OF AWARD:				1982		100	
E. AMOUNT:							
F. CUM. AMT.							
21. RESPONSIBLE S&T ORGANIZATION				22. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Precede with U.S. Academic Institution)			
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TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2968			
23. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Precede individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>(U) Resuscitation Fluids; (U) Burn Injury; (U) Cardiac Output; (U) Cardiovascular Hemodynamics; (U) Indicator Dilution; (U) Humans; (U) Animal Model</p> <p>output (FA) thermal curves, were dissimilar as flow increased, indicating that the thermal tracer is diffusion limited by the short transit times at high flows. The data suggest that the thermal-green dye technique may underestimate lung water in burned and other critically ill patients with hyperdynamic circulations.</p>							

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

**REPORT TITLE: THE CARDIOPULMONARY RESPONSE TO THERMAL INJURY IN
BURNED SOLDIERS - UNDERESTIMATION OF THERMAL
LUNG WATER VOLUME IN HIGH CARDIAC OUTPUT PATIENTS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**Cleon W. Goodwin, Jr., MD
Basil A. Pruitt, Jr., M.D., Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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US Army Institute of Surgical Research, Brooke Army Medical
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Period covered in this report: 1 October 1980 - 30 September
1981

Investigators: Cleon W. Goodwin, Jr., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

When utilizing the intravascular double indicator dilution technique to measure extravascular lung water, blood flow may be so high that diffusion equilibrium of the diffusible indicator fails to occur and the water distribution space is underestimated during the first seven days following thermal injury. We serially measured cardiac index and lung water in five severely burned patients (mean age 24 years, range 18 to 33 years; mean burn size 56% TBS, range 43 to 80%) by a rebreathing method utilizing two gases of differing solubility and by the thermal-indocyanine green dye (ICG) double indicator dilution technique. Rebreathing lung water, determined by a time and blood flow insensitive method, increased significantly over the study period, from 6.6 ml/kg on admission to 11.3 ml/kg on post-burn day 6 (+70%, $p < 0.01$). Thermal-ICG lung water slightly decreased as blood flow rose. Rebreathing lung water correlated with clinical data in a patient with pulmonary edema, while thermal-ICG technique may be diffusion limited by short transit times at the high flows characteristic of burned and other critically ill patients with hyperdynamic circulations. Additionally, segmental redistribution of pulmonary blood flow known to occur in burn patients may contribute to underestimation of lung water.

Lung water
Cardiac output
Resuscitation
Pulmonary edema

**THE CARDIOPULMONARY RESPONSE TO THERMAL INJURY
IN BURNED SOLDIERS - UNDERESTIMATION OF THERMAL
LUNG WATER VOLUME IN HIGH CARDIAC OUTPUT PATIENTS**

Following massive thermal injury, the lung participates in the pathophysiological response associated with large plasma volume loss and administration of large resuscitation volumes, i.e., the formation of tissue edema in the area of injury and probably in noninjured tissues (1). Pulmonary injury is frequently associated with large cutaneous burns, up to 25% of patients admitted to our treatment facility (2-4), accentuates the fluid requirements during resuscitation, and predisposes to the development of acute pulmonary edema during the first postburn week (5, 6). Occasionally, early pulmonary edema with coexisting inhalation injury or preexisting cardiovascular disease occurs when peripheral edema in the burn wound is rapidly mobilized. Because most pulmonary dysfunction during the early phase of burn injury results either directly or indirectly from abnormal distribution and accumulation of lung tissue water, the ability to measure extravascular lung water (EVLW) serially not only would help elucidate the magnitude and direction of acute fluid shifts and the effects of hypoproteinemia but also would help identify the optimal volume and composition of the resuscitation fluid. Several preliminary studies of diverse groups of burned patients have indicated that passive lung water changes are related to coexisting pulmonary injury or sepsis and are not directly dependent on plasma oncotic forces (7-9). Furthermore, the addition of colloid to balanced electrolyte solutions

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during resuscitation to maintain normal plasma oncotic pressures does not appear to limit the accumulations of excess lung water and may, in fact, produce the opposite effect (10,11).

Chinard and co-workers laid the theoretical basis and introduced the first practical method for the *in vivo* measurement of extravascular lung water (12-15). In principle, following bolus injection of a solution containing a nondiffusing intravascular tracer (e.g., labelled red blood cells) and a diffusible water tracer (e.g., THO) into the arterial inflow of the lungs, the venous concentration-time curves of the tracers reflect the individual distribution spaces of the two tracers. The volume of each distribution space is equal to the flow through the lungs and the mean transit time of each tracer. Since flow for both tracers is equal, the extravascular volume can be calculated as the product of flow times the difference between mean transit time for the water space (which is composed of both intravascular and extravascular pools) and the mean transit time for the intravascular pool. This volume, in theory, represents EVLW. The initial animal studies were extended to human studies, and the results roughly paralleled clinical findings in various disease states, establishing the utility of this method for use in patients (16,17).

The use of an isotope of water as the water tracer has been found to underestimate EVLW, especially when used in the presence of lung edema, because of the lack of equilibrium of such tracers with all tissue water within one passage through the lung. The use of a thermal indicator with in-stream thermistor tipped catheters has been shown by concomitant gravimetric analysis to measure EVLW

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more accurately (18-20). More recently, a portable bedside microprocessor has been developed and extensively utilized, which allows rapid calculation of EVLW and cardiac output (21). Postmortem estimation of lung water was in good agreement with antemortem EVLW measurements in animal studies, and similar measurements in patients are reported to correlate with their clinical conditions.

Lung water can also be measured by a rebreathing method utilizing two gases of differing solubility. Tissue volume determinations have been shown to reflect lung water changes (with reliability) in animals with normal and edematous lungs (22). The theoretical basis and original breath-holding technique were described by Cander and Forster (23). Subsequent modifications to allow rebreathing of the tracer gases facilitate clinical application of this technique (24). When a soluble gas is inspired into the lungs, equilibration between the alveolar gas and the surrounding lung tissue volume occurs within approximately 10 milliseconds. The dissolution of soluble gas into the lung tissue volume results in an initial fall in gas concentration, and its magnitude is a direct function of the tissue solubility of the gas and the volume of lung tissue. In the subsequent 15 to 20 seconds, the concentration of soluble gas in the alveoli decreases exponentially as it equilibrates with, and is transported away by, the pulmonary capillary circulation. By obtaining serial alveolar gas samples, a disappearance curve can be constructed, and when plotted semilogarithmically, the intercept and slope reflect lung tissue volume and pulmonary capillary blood flow, respectively.

The principle advantage of the rebreathing method is that it is noninvasive. In addition, with suitable gases, additional physiologic indices of pulmonary function can be calculated from the gas measurements. However, the

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rebreathing method requires very expensive equipment, complicated data reduction, and, in our hands, alert and spontaneously breathing patients. Although the intravascular double indicator dilution method requires access both to the pulmonary artery and to a systemic artery, current hardware allows sterile sampling without loss of blood from the patient (21). In addition, serial measurements are quite easy to perform in all patients, including those being treated with mechanical ventilators. This study evaluates the utility of these two independent methods for quantification of EVLW and defines the limitations of each in patients with massive thermal injuries.

MATERIAL AND METHODS

Subjects. Five thermally injured patients who required hemodynamic monitoring with pulmonary arterial and systemic arterial catheters were serially studied after obtaining informed consent for participation in a research protocol approved by institutional review (Table 1). All were admitted within 12 hours of injury; their mean age was 24 years (range 18 to 33 years) and mean burn size, 56% of the total body surface (range 43% to 80%). During the first 24 hours, the patients received lactated Ringer's solution at a rate sufficient to stabilize vital signs and to achieve a urinary output of 30 to 60 ml per hour. Utilizing these guidelines, the mean resuscitation volume in the 24 hours after injury was 3.45 ml/kg body weight/percent body surface burn (range 2.80 to 4.36 ml/kg % burn). Plasma volume was replaced on the second postburn day by colloid equivalent to plasma in a dosage of 0.3 to 0.5 ml/kg body weight/percent body surface burn. Following the initial 24-hour resuscitative phase, 5% dextrose in water was administered at a rate which allowed the patient's weight to return to preburn levels by postburn day 7 to 10 and which maintained serum sodium and osmolal concentrations in the normal range. No patients had accompanying inhalation injury or other pulmonary disease based on clinical evaluation and on normal xenon ventilation-perfusion lung scan, fiberoptic bronchoscopy, chest roentgenogram, and arterial blood gases. None of the patients developed positive blood cultures or demonstrated microbiological or clinical evidence of pulmonary infection during the seven days of the study. An additional patient, who sustained an inhalation injury and subsequently developed early pulmonary edema, was studied under a separate protocol and is discussed to illustrate water changes in injured lungs.

Study Design. Studies were carried out every 12 hours (0600 and 1800 hours) for the first three postburn days and daily (0600 hours) on postburn days 5, 6, and 7. Patients were studied in a semi-recumbent position to which they had acclimated for several hours prior to study. EVLW was measured by two independent methods: a rebreathing method utilizing two gases of differing solubility and an intravascular double indicator dilution technique. The order in which the two methods were employed was randomized for each study.

Table I. Patient characteristics and summary of selected clinical data during initial postburn week

Patient	Age (Years)	Sex	TBS/3 ⁰ Burn (%)	Resuscitation (ml/kg/3 TBS)	Preburn Weight (kg)	Maximal Weight Gain (PBD) (%)	Chest Roentgenograms Normal	Inhalation Injury	Lowest Room Air P_{aO_2} (torr)	Outcome
1	33	M	50/17	4.12	66	19(2)	Yes	No	90	Survived
2	18	M	80/55	2.80	95	12(2)	Yes	No	79	Survived
3	20	M	43/28	4.36	66	12(2)	Yes	No	92	Survived
4	19	M	55/46	2.96	66	4(1)	Yes	No	89	Survived
5	28	M	53/39	3.01	80	8(1)	Yes	No	*	Died (PBD 29; of sepsis)

LEGEND:

TBS - total body surface

PBD - postburn day

* P_{aO_2} was measured in this patient with face mask and humidified oxygen at FI_{O_2} -0.36; the lowest P_{aO_2} under these conditions was 134 torr.

Rebreathing lung water. The distribution of each test gas (helium and dimethyl ether, DME) was measured by a time of flight medical mass spectrometer (MGA 1100A, Perkin-Elmer Corp.). Modifications to the mass spectrometer include a heated stainless steel capillary inlet and appropriate mass plates (helium=4 and DEM=15). At a gas sampling rate of 60 ml per minute, the inlet system introduced a sampling time delay of approximately 300 ms. Response time (95 percent) of the mass spectrometer was 60 ms. A bag-in-box with a 16 inch spring-loaded gas impermeable low compliance reservoir bag (Calibrated Instruments, Inc.) was connected with large bore tubing to a previously calibrated data acquisition dry spirometer (843, Ohio Instrument Co.) for a volume signal output. A three-way pulmonary breathing valve (Hans Rudolph, Inc.) with a mouthpiece selects room air or the rebreathing bag. A fiberoptic recorder (1858, Honeywell, Inc.) with a frequency response of 5000 hz recorded the electrical output of the helium, dimethyl ether, and bag volume signals. The initial bag volume was adjusted with the test gas mixture of 1.5% DME, 7% helium, 30% oxygen, and balance nitrogen to approximate the one second forced expiratory volume (three liters). The subject with nose clip in place was asked to breathe quietly through the mouthpiece. He was then instructed to exhale to residual volume, the valve turned into the rebreathing bag, and verbally directed consecutive maximal rebreathing maneuvers were carried out for 15 to 20 seconds.

The signal tracings and calibration standards were digitized off-line from the photographic paper onto a mini-computer (9830, Hewlett Packard, Inc.), which corrected the raw data for time of passage of gases through the sampling system for gas consumption by the mass spectrometer (60 ml/min), and for anatomic and apparatus dead space in the first end-expiratory volume cycle. The disappearance of the soluble gas, DME, is plotted on semilogarithmic paper so that its slope (pulmonary capillary blood flow) and its time zero intercept (tissue volume) can be calculated. To detect tracer gas recirculation, which is indicated by a decrease in the logarithmic washout slope, serial least squares lines were calculated through at least three of the first six rebreathing points and the time zero intercept. The line yielding the best squared correlation coefficient was chosen for subsequent calculations. Calculated values include lung tissue volume (lung water), residual lung volume, alveolar volume, pulmonary capillary blood flow (cardiac output), and rebreathing dead space (24). Rebreathing lung water results are expressed as ml/kg of preburn weight. All measurements were made in duplicate. Intervals of at least five minutes between each study were observed to allow exhalation of any soluble gas that may have accumulated in the body.

Thermal ICG lung water. Ten ml of iced 5% dextrose solution containing 10 mg of indocyanine green (ICG, Hynson, Westcott, and Dunning, Inc., lot #386) were injected by a CO₂ gas injector (37200, USCI Cardiology Products, Inc.) through the proximal port of the pulmonary artery catheter into the right atrium. ICG concentration was measured by sterily withdrawing blood through the thermistor-tipped femoral artery catheter (96-020-5F, Edwards Laboratories, Inc.)

and a disposable cuvette (9602, Edwards Laboratories, Inc.) connected to an ICG densitometer (DCR-702, Waters Instruments, Inc.). At the completion of each measurement, the withdrawn blood was reinfused into the patient. The ICG and the femoral artery thermal signal were detected and digitized automatically by a portable microprocessor (9310, Edwards Laboratories, Inc.). Extravascular lung water is calculated as the product of thermal dilution cardiac output and the difference in mean transit times of the thermal and ICG curves. Five consecutive lung water measurements were recorded at each study period, and their mean was expressed in ml/kg of preburn weight.

Statistical analysis. A one-way analysis of variance was used to examine serial changes within each technique group. A two-way analysis of variance was utilized to detect differences between the two technique groups. Statistical differences with $p < 0.05$ were accepted as significant. Values are reported as mean - standard error.

RESULTS

Table 2 summarizes the changes during the seven day study in rebreathing lung water, thermal-ICG lung water, cardiac index, and the mean transit times for the ICG and thermal indicators. Lung water measured by the rebreathing technique increased significantly by completion of the study, with a maximal increase of +70 percent on postburn day six ($F=3.55$, $p < 0.01$) (Fig. 1).

Lung water measured by the thermal-green dye technique decreased slightly by postburn day seven (-20%), but this change was not statistically significant ($F=1.498$, $p > 0.05$). The two technique groups were statistically distinct from one another ($F=35.6$, $p < 0.001$); however, since each method measures a physically different distribution property, the statistical comparison of the results of each method with the other may be artificial.

Cardiac index rose progressively in all patients as postburn hypermetabolism developed during the first postburn week ($F=8.927$, $p < 0.001$). The rising flow was reflected by shortening of the mean transit time of the intravascular ICG and diffusible thermal indicators.

The use of a gas syringe injector for the bolus injection of the chilled ICG solution produced the most uniformly reproducible results. Using this technique, the coefficients of variation for computing extravascular lung water was 9.8%, for cardiac output, 7.9%, for ICG mean transit time, 4.9%; and for thermal mean transit time, 6.6%. Hand injections produced large variations in indicator mean transit times during serial measurements but resulted in minimal variability in the computed cardiac outputs and lung water determinations.

In a patient who sustained inhalation injury (not included with the data of the study patients), lung waters measured by each method changed in opposite directions during an episode of fluid overload and acute pulmonary edema in the presence of an elevated cardiac output (Fig. 2). When pulmonary edema became clinically evident, lung water determined by the thermal-ICG technique decreased, while that measured by the rebreathing method increased. These changes were paralleled by a moderate increase in pulmonary shunt and roentgenographic

Table 2. Serial changes in cardiac output and lung water following thermal injury.

Postburn Day	0.5	1.0	1.5	2.0	2.5	3.0	5	6	7
CI (L/min/m ²)	3.44 ±0.23	3.12 ±0.47	4.75 ±0.55	4.71 ±0.20	4.99 ±0.52	5.66 ±0.75	6.55 ±0.44	7.15 ±0.43	6.75 ±0.46
RBLW (ml/kg)	6.55 ±0.87	7.16 ±0.91	7.87 ±0.58	7.76 ±0.65	7.17 ±0.61	8.24 ±1.14	8.84 ±1.06	11.25 ±1.65	10.39 ±0.62
TGLW (ml/kg)	5.32 ±0.82	4.95 ±0.67	4.81 ±0.61	4.44 ±0.55	4.68 ±0.62	4.76 ±0.58	3.86 ±0.34	4.10 ±0.44	4.53 ±0.46
MTT ₁ (sec)	4.18 ±0.33	4.33 ±0.22	3.52 ±0.22	3.55 ±0.22	3.37 ±0.27	3.19 ±0.19	3.05 ±0.14	3.06 ±0.22	3.36 ±0.23
MTT ₂ (sec)	8.07 ±0.51	7.69 ±0.32	6.00 ±0.04	5.81 ±0.37	5.40 ±0.58	5.52 ±0.61	4.44 ±0.15	4.48 ±0.23	4.92 ±0.23

Legend: CI - cardiac index, RBLW - rebreathing lung water, TGLW - thermal-ICG lung water, MTT₁ - mean transit time for ICG, MTT₂ - mean transit time for thermal indicator. Values represent mean ± SE.

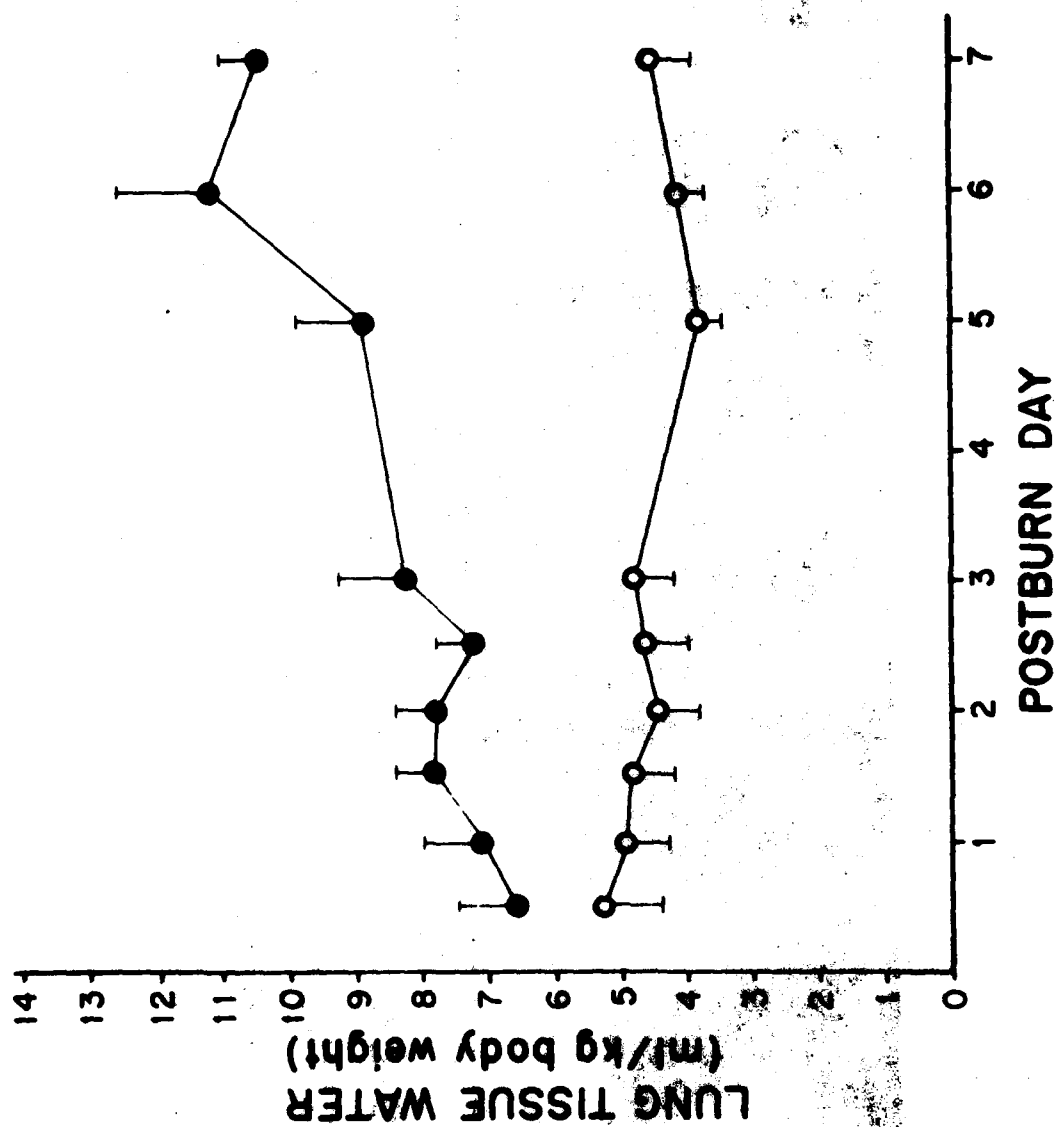
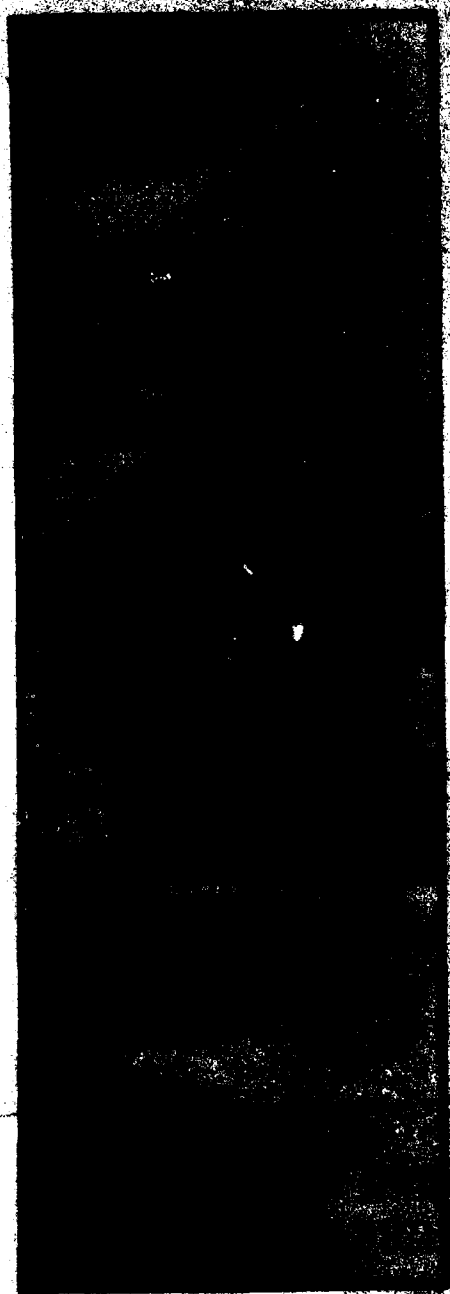


Figure 1. Time course of changes in lung water measured by the rebreathing method (-●-) and by the thermal-ICC method (-○-). Values are normalized to preburn weight.



CO (L/min)	12.30	14.40	13.20
TDLW(ml/kg)	6.81	5.81	6.45
RBLW(ml/kg)	10.58	13.41	8.57
Q _s /Q _T (%)	10.00	18.00	8.00

Figure 2. Changes in blood flow, intrapulmonary shunt, and lung water on PBD 5, 6 and 7 in a 27 year old patient with a 60 per cent TBS burn and inhalation injury (CO - cardiac output, TDLW-thermal-ICC lung water, RBLW rebreathing lung water, Q_s/Q_T shunt).

evidence of pulmonary fluid overload. With fluid restriction and diuretics, all measurements changed in the opposite direction: thermal-ICG lung water increased, rebreathing lung water decreased, pulmonary shunting lessened, and the chest roentgenogram cleared.

DISCUSSION

During the initial week following large thermal injuries, EVLW determined by a well-standardized rebreathing method increased significantly in our patients. This increase in lung water was modest during the first three post-burn days, a period when intravascular volume deficits were being corrected and injured tissue accumulated large volumes of edema fluid. During the subsequent four days, the increase in EVLW became much more pronounced and was associated in time with decreased fluid requirements and mobilization of the massive quantity of burn wound edema. By contrast, EVLW as measured by the intravascular double indicator dilution technique (thermal-ICG) did not change significantly during the seven day study period and, if anything, demonstrated a tendency to decrease during that interval. In large reported series, clinical pulmonary edema in the early postburn period occurs most frequently between the third and seventh postburn days (4,5) and this pattern corresponds with the period of greatest lung water accumulation detected by the rebreathing technique in our patients, who had no evidence of acute pulmonary injury. In an additional patient with inhalation injury (not included in the main study group) who was evaluated by both measurement techniques, the clinical, physiologic, and roentgenographic evidence of pulmonary edema correlated directly with the rebreathing lung water and inversely with that determined by the intravascular method. The greatest divergence of the two techniques occurred during the latter half of the study interval, when cardiac output was markedly elevated and mean transit times for the indicators quite shortened. These physiologic conditions may impose limitations on the intravascular method when utilized in patients with hyperdynamic circulations.

The validity of the rebreathing method for estimating lung water deserves careful examination. Strictly speaking, the volume in which the soluble gas tracer distributes during breathing measures lung tissue volume, not water volume, which can be truly measured only with water as the tracer molecule. However, water comprises over 80 percent of the lung tissue volume (23), and thus the majority of the tissue volume measurement reflects lung water content. More importantly, during serial studies over several days, the solid tissue structures of the lung can be assumed to remain constant, and any changes in measured lung tissue volume likely represents change in that organ's water content. For this reason, this technique has been proposed as an effective method for noninvasively monitoring lung water changes during certain acute diseases and following therapeutic interventions.

The accuracy of measurements of lung tissue volume as a reliable indicator of water content has been verified in animal studies. Gravimetric analysis of lung water correlates closely with rebreathing measurements until lung weight

increases in excess of 250 percent of control values (22,25-28). Beyond this point, alveolar flooding occurs, with massive obliteration of alveolar air spaces and restriction of soluble gas distribution (29). The validity of tissue volume measurements for detecting lung water in humans is more difficult to verify, since gravimetric analysis of lungs obtained long after death probably do not accurately reflect *in vivo* circumstances. Increased lung tissue volume has been documented in patients with clinical pulmonary edema (30), and it is likely that the rebreathing method is as valid in humans as it is in animal models (31).

The major criticism of the rebreathing method is that it measures not only the water in the pulmonary interstitial tissues but also that in the capillaries. In our study, we did not independently measure pulmonary capillary blood volume, as can be done while determining carbon monoxide diffusing capacity (32). However, we can indirectly assess the contribution of pulmonary capillary blood volume to the estimates of lung water in our patients. Central blood volume can be calculated from the cardiac outputs and the mean transit times of the intravascular indicator. In our patients, the increase in central blood volume from the time of intravascular volume restitution (postburn day three) to time of maximal lung water (postburn day six), at most, can account for 50 percent of the approximately 375 ml mean increase in lung water of each patient. However, central blood volume includes all the blood between the tip of the injection catheter and the densitometer, not only the pulmonary capillary blood volume, but that in the right ventricle, pulmonary arteries and veins, left side of the heart, and aorta. Pulmonary vascular volume is slightly smaller than

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central blood volume with seventy-two percent of the pulmonary blood volume present in the first five orders of branching from the main pulmonary artery (33). Both of these calculated blood volumes greatly overestimate the contribution of the pulmonary capillary blood volume.

The pulmonary capillaries of adults contain 80 to 100 ml of blood under normal conditions (23,34,35). Calculated central blood volumes increased as cardiac outputs rose in our patients. Pulmonary dispersive volume, reflecting total pulmonary vascular volume, has also been noted to be directly related to cardiac output (36,37). Serial measurements of lung tissue volume, capillary blood volume, and cardiac output with graded exercise in human subjects have demonstrated that a near doubling of cardiac output was associated with an increase of pulmonary blood volume from 101 ml to 123 ml (28). Moreover, pulmonary tissue volume measurements were not correlated with blood flow. In animal studies, large vessel blood volume was found to increase massively when acute pulmonary edema was induced by elevating left atrial pressure, while at the same time, pulmonary capillary blood volume, measured by carbon monoxide diffusion, increased only transiently, and returned to baseline within a few hours (38). From the above considerations, it is unlikely that an increased pulmonary capillary blood volume contributed significantly to the increased tissue volume measured in our patients. Thus, assuming the capillary volume remained unchanged at 100 ml, lung tissue volume increased 90 percent and most likely represents water accumulation. If a "worst case" is considered, i.e., a doubling of lung capillary blood volume, lung tissue volume still increased 65 percent.

While calculated lung tissue volume is minimally affected when blood flow is unevenly distributed, it is significantly underestimated when ventilation is unevenly distributed (24). We have attempted to avoid this consideration by studying a homogeneous group of patients with known normal lung function. The

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effect of any nonventilated lung segments is eliminated in large part by normalizing the measured lung water (tissue volume) to measured lung volumes. This procedure not only takes into account only those areas of the lung into which the soluble gas diffuses but also allows comparisons of individuals of different sizes. Normalizing lung tissue volume in this manner renders the rebreathing technique for lung water estimation a reliable, highly reproducible method in both normal and moderately edematous lungs (10,26,27,30). If the data from our patients are normalized to alveolar volume (our usual method) instead of body weight, the increase in lung water over the seven day study is even more highly significant.

Other sources of error are introduced by recirculation of the soluble gas indicator, change in alveolar volume due to uptake of O_2 by the blood, and undetected dead space in the measuring system. Since the slope of the soluble gas washout determines the time of intercept and hence, the lung tissue volume measurement, recirculation of the indicator gas would decrease that slope and overestimate the tissue volume. With moderate exercise, the recirculation time of nitrous oxide decreases from 15 seconds at rest to 8 seconds (39). Burned patients with hyperdynamic circulations show a similar shortened circulation time, and we are able to detect recirculation by the least squares analysis of sequential points on the washout slope, as described above. Although alveolar volume, an important component of the calculation of lung tissue volume, is assumed to be constant, it actually decreases due to uptake of O_2 from the alveolus by the blood. However, for normal subjects this change in alveolar volume introduces an error in tissue volume of less than one percent (24), and with twice normal O_2 consumption, a typical situation in hypermetabolic burn patients, such error would alter tissue volume by less than two percent. Finally, dead space and attendant incomplete and delayed mixing of gases can be minimized by beginning the testing procedure at residual volume and by utilizing near maximal rebreathing maneuvers (3 liters), as we describe above.

As with the rebreathing method, the diffusible thermal indicator of the intravascular indicator dilution method does not detect lung water volume. Rather, the thermal tracer detects a thermal volume which includes not only parenchymal lung tissue structures and water content, but also the thermal distribution of the left side of the heart, the bronchi, pulmonary arteries and veins, and possibly a small portion of the chest wall (40). These latter components may contribute as much as 30 to 35% of the measured tissue volume under basal conditions. The degree to which thermal volume measurements correspond to EVLW depends in part on the intravascular reference indicator. When hypertonic saline is employed, lung thermal volume overestimates gravimetric lung water 4 to 20 percent (18,20). Albumin labelled with ICG, currently the most widely used reference tracer, yields lung water measurements which either moderately underestimate or correlate quite closely to that obtained from gravimetric analysis.

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This close correlation holds true not only under normal conditions but also in the presence of pulmonary edema, emboli, and sepsis (41-46).

The reasons why the thermal ICG technique may have underestimated lung water in our patients are not clear. Immediately after burn injury, before large resuscitation volumes had been administered, we measured a mean thermal volume of 5.3 ml/kg. This value is entirely consistent with thermal ICG lung water of 5.7 ml/kg measured in similar patients without pulmonary pathology (21). However, in the ensuing six days, thermal-ICG lung water fell to a mean of 3.9 ml/kg on postburn day five in our patients, with values less than 3 ml/kg in some patients. This low quantity of lung water is physiologically untenable, and we have examined potential sources of error in the technique which may explain these circumstances.

The use of albumin alone as the reference tracer for the intravascular distribution of water can result in sizeable underestimation of EVLW (47). The reflection coefficient of albumin is not unity, and any leak of albumin out of the circulation and into the interstitial spaces will alter the distribution of its transit times and will increase its mean transit time and, thus, its apparent intravascular volume of distribution. In addition, plasma separates from red cells in small vessels, creating an intravascular plasma space which is accessible to albumin but not to red cells (48). This separation becomes more pronounced with increased blood flow and with edema (49). However, since

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water distributes in both red cells and in plasma, the intravascular transit time used for EVLW calculations must take this into account. To use only the albumin transit time will lead to overestimation of the intravascular water volume and net underestimation of EVLW. This discrepancy increases with rising cardiac output and with large changes in hematocrit, both of which occur during the first postburn week.

Various components of the equation for computing thermal dilution cardiac output may also be inaccurate. The catheter correction factor is dependent not only on catheter volume but also on hematocrit and blood flow. Heat loss from the catheter causes overestimation of the calculated cardiac output and is dependent on blood flow (50,51). These inaccuracies are more pronounced at low flows and tend to be minimized at higher blood flow. Furthermore, recirculation may cause underestimation of cardiac output. Since the bedside computer utilized in this study analyzes the concentration-time curves out to a set fraction of the peak concentration values, recirculation, especially under conditions of very rapid transit time, may be undetected.

Heat is advanced as a more ideal tracer than labelled water for determining EVLW because its diffusivity is 100 times greater than water (52). However, this very quality may limit its use in the indicator dilution measurements. Because it does not distribute by the same mechanisms as molecular tracers, the use of blood flow as the flow factor for the thermal tracer may not be applicable. Thermal volume calculated from blood flow is not synonymous with that calculated from heat flow. As volume and velocity of blood flow increase, the relationship between blood flow, heat flow, and thermal volume may change, and may explain the very short thermal mean transit times and small thermal volumes in our patients (53). Higher heat flows relative to blood flow may explain why the thermal curves preceded the ICG curves, especially at lower flows, in our patients. Finally, like tritiated water, heat capacity may be diffusion limited at higher blood flows (54).

Since the marked decline in thermal volume occurred in our patients only after cardiac output had doubled, diffusion limitation and underestimation of EVLW may take place at a threshold flow value, below which EVLW is not affected by blood flow (43). Another factor which may contribute to the

50. Maruschak BG, Potter AM, Schauble JF, Rogers MC: Overestimation of pediatric cardiac output by thermal indicator loss. *Circulation* 65:380-3, 1982

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54. Staub NC: Pulmonary edema. *Physiol Rev* 54:678-811, 1974

discrepancy between the measurements of lung water is the fact that the two methods measure lung water from opposing sides of the alveolar-capillary membrane. The thermal-ICG technique can "look" only at that lung water with which it makes contact. Impaired perfusion of lung tissue, known to occur in burn patients (55), will reduce the volume of lung water into which the thermal indicator can diffuse while the diffusible gas will still make contact with such water as long as the alveoli in such poorly perfused segments of the lung are ventilated.

Overall, the rebreathing technique appears to be a reliable method for determining EVLW even in patients with markedly elevated cardiac output. Serial changes in lung tissue volume, measured by this method, likely reflect alterations in lung tissue water. If anything, this technique may underestimate EVLW, especially when a very soluble gas, such as dimethyl ether, is used. Under normal conditions and in a variety of pathological circumstances, the thermal-ICG technique has also been shown to be very dependable and reproducible and has, in addition, specific operational advantages. However, the thermal-ICG method appears to underestimate EVLW in patients with markedly elevated cardiac outputs, and this technique may require modification when utilized in burned or other critically ill patients with hyperdynamic circulations. The differences in EVLW as determined by the two methods may in some manner be related to intrapulmonary shunt flow. Simultaneous use of the two techniques may permit estimation of that volume of lung tissue to which circulation is impaired.

55. Cook WA, Baxter CR, Ferrell JM: Pulmonary circulation after dermal burns. *Vasc Surg* 2: 1-11, 1968

PRESENTATIONS:

Goodwin CW: Underestimation of thermal lung water volume in high cardiac output patients. Presented at the meeting of the Society of University Surgeons, New York, N.Y., 13 February 1982.

PUBLICATIONS:

Goodwin CW, Pruitt BA, Jr.: Underestimation of thermal lung water volume in high cardiac output patients. Accepted for publication in *Surgery*, 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION#	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL	
				DA OG 6971	81 10 01	DD-DR&E(AR)436	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DES'N INST'N	9. SPECIFIC DATA- CONTRACTOR ACCESS	
80 10 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES:		PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY		62772A	35162772A874	AF	162		
b. XXXXXXXX							
c. XXXXXXXX		STOG 80-7.2:5					
11. TITLE (Precede with Security Classification Code)							
(U) Evaluation of Burn Wound Care in Troops With Burn Injury (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
76 10		Cont		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
Not Applicable				PRECEDING			
a. DATES/EFFECTIVE:				FISCAL YEAR		b. FUNDS (in thousands)	
EXPIRATION:				1981		2.0	
b. NUMBER:				CURRENCY		74	
c. TYPE:				1982		1.5	
d. KIND OF AWARD:				f. CUM. AMT.		60	
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Clinical Division Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr., MD, COL, MC				NAME: William F. McManus, MD, COL, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-3301			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
22. REFERENCES (Precede EACH with Security Classification Code)							
(U) Burn Injury; (U) Topical Therapy; (U) Sulfamylon; (U) Wound Excision; (U) 5% Sulfamylon Acetate Solution; (U) Humans; (U) Autografts							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) The military relevance of improving burn wound care will be realized in increased troop survival following thermal injury. Newer methods under current investigation include the use of 5% aqueous Sulfamylon soaks, excision of eschar from burned soldiers, planned evaluation of cerium silver sulfadiazine, and the use of frozen homografts in burn wound care. Our objective is to further define the use of these methods.</p> <p>24. (U) Patients admitted to the Institute of Surgical Research for care of thermal injuries receive burn care based on the specific injury. The 5% aqueous Sulfamylon soaks, excision of the eschar, and other modalities of wound care may be used.</p> <p>25. (U) 8010 - 8109. Treatment with 5% aqueous Sulfamylon was utilized in 136 patients. Eight patients (5.9%) exhibited some form of allergic reaction. These 8 patients required no treatment of mild atopy. The low incidence of reactions and the clinical effectiveness of 5% aqueous Sulfamylon speaks for its continued use. Standard topical antimicrobial therapy of the burn wound continues to be the sequential application of mafenide acetate and silver sulfadiazine every 12 hours to maximize the spectrum of antibacterial effectiveness and minimize the side effects of the respective agents. The indications for excision of the burn wound continue to include deep dermal hand burns that will not heal in three weeks, sequential excision of full thickness burns limited to 20% of the total body surface at any one procedure, removal of documented wound infection and debridement of retained non-viable tissue.</p>							

DD FORM 1498
1 MAR 68

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ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH
BURN INJURY: 5% AQUEOUS SULFAMYLON SOAKS USED IN
TOPICAL TREATMENT OF BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROCKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1980 - 30 September 1981

Investigators:

William F. McManus, M.D., Colonel, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

**REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH
BURN INJURY: 5% AQUEOUS SULFAMYLON SOAKS USED IN
TOPICAL TREATMENT OF BURNED SOLDIERS**

**US Army Institute of Surgical Research, Brooke Army Medical
Center, Fort Sam Houston, Texas 78234**

Period covered in this report: 1 October 1980-30 September 1981

**Investigators: William F. McManus, M.D., Colonel, MC
Basil A. Pruitt, M.D., Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

The use of 5% aqueous Sulfamylon dressings in the care of the burn wound has continued to be an efficacious treatment modality throughout this report period. A hundred and thirty-six patients were treated with 5% aqueous Sulfamylon dressings employed either for final debridement of a wound or following application of meshed cutaneous autograft to prevent desiccation of tissue exposed in the interstices of such grafts. A 5.9% incidence of skin rash (atopy) was noted as the only adverse reaction. The clinical results achieved by the use of 5% aqueous Sulfamylon solution support its continued use.

**Burn injury
Topical therapy
5% Sulfamylon acetate solution
Humans**

**EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: 5%
AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF BURNED
SOLDIERS**

During the reporting period of 1 October 1980 through 30 September 1981 evaluation of 5% Sulfamylon acetate solution for topical treatment of the burn wound has continued at this Institute and involved its use in 136 (65%) of the 209 patients admitted to the U.S. Army Institute of Surgical Research. During this period 359 split thickness autograft procedures were performed in 131 patients; 5% aqueous Sulfamylon soaked dressings were used in conjunction with the skin autografting procedures in 119 patients. The 5% Sulfamylon acetate soaked dressings are used as wet to dry dressings to debride nonviable tissue elements in preparation for split thickness autograft procedures or as continuous wet dressings to protect freshly excised wounds that are not autografted. In addition when meshed cutaneous autografts are applied dressings are soaked with 5% Sulfamylon acetate to decrease the rate of bacterial growth and to prevent desiccation of tissue exposed in the interstices of such grafts.

Eight patients (5.9%) demonstrated allergic reactions (atopy) coincident with the use of 5% aqueous Sulfamylon solution and these eight patients demonstrated rapid resolution of the atopic reaction following administration of an antihistamine and/or discontinuation of the 5% aqueous Sulfamylon soaked dressings. Saline or other aqueous topical antimicrobial agents were substituted once 5% aqueous soaked Sulfamylon dressings were discontinued and no other adverse reactions were noted in this group of patients.

The continued use of 5% aqueous Sulfamylon acetate dressings has been efficacious both in the preparation of the burn wound for cutaneous autografting and in the prevention of desiccation of ungrafted granulation tissue. This efficacy and the low incidence of adverse side effects speak for continued use of this solution.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(A/R)55	
3. DATE PREV SUMMARY 80 10 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY ^a U	6. WORK SECURITY ^a U	7. REGRADING ^a NA	8A. DISSEM INSTR ^a NL	8B. SPECIFIC DATA, CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	8C. LEVEL OF SUM A. WORK UNIT
10. NO./CODES: ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		62772A		35162772A874		AF 163	
b. XXXXXXXXXX							
c. XXXXXXXXXX		STOG 80 - 7.2:5					
11. TITLE (Precede with Security Classification Code) ^a							
(U) Studies of The Neuroendocrine Abnormalities in Burn Injury (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE SERVICES	
79 10		Cont		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
Not Applicable				PREVIOUS		2.0	
a. DATES/EFFECTIVE:				FISCAL		1981	
b. NUMBER: ^a				YEAR		CURRENT	
c. TYPE:				1982		1.7	
d. KIND OF AWARD:				93			
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a U.S. Army Institute of Surgical Research				NAME: ^a US Army Institute of Surgical Research			
ADDRESS: ^a Ft Sam Houston, Texas 78234				ADDRESS: ^a Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr., MD, COL, MC				NAME: ^a George M. Vaughan, MAJ, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-5416			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
22. KEY WORDS (Precede with Security Classification Code) ^a							
(U) Pineal; (U) Hypothalamus; (U) Thyroid; (U) Indoles; (U) Catecholamines							
(U) Laboratory Animal; (U) Human Volunteer							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To determine the hormonal abnormalities in burned soldiers.							
24. (U) The initial approach was to assess the neurological basis for the cortisol and melatonin rhythms in non-burned humans and determine the effects of acute stress on these hormones. Plasma melatonin was investigated as an index of pineal function. Next, the relationships among plasma cortisol, ACTH, core temperature, metabolic rate and catecholamine excretion were determined in burned soldiers, and characteristics of the cortisol rhythm were also determined in such patients. The effect of a 37% burn on morning corticosterone, ACTH and thyroid hormone levels was determined in adult male rats.							
25. (U) 8010 - 8109. The human plasma melatonin rhythm behaves as predicted from known pineal physiology in experimental animals, including dependence of the nocturnal surge on an intact hypothalamus. Acute stress does not perturb melatonin levels. In burned soldiers, morning cortisol (and not ACTH) was elevated in proportion to burn size, and hypermetabolism and hyperthermia were more closely related to urinary catecholamine excretion than to plasma cortisol. The cortisol rhythm mean was markedly elevated, and the amplitude was suppressed. Melatonin levels will be assessed in subsequent studies of burn patients. In burned rats on postburn days 1, 3 and 7, corticosterone was elevated, and T ₄ and T ₃ levels were suppressed, in agreement with previous human data. Thus, the rat burn model may provide a reasonable approximation to the human burn disease.							

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ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

**PROJECT TITLE: STUDIES OF NEUROENDOCRINE ABNORMALITIES
IN BURN INJURY - CORTISOL AND CORTICOTROPHIN
IN BURNED SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**George M. Vaughan, M.D., Major, MC
Richard A. Becker, M.D.
John P. Allen, M.D.
Cleon W. Goodwin, Jr., M.D.
Basil A. Pruitt, Jr., M.D.
Arthur D. Mason, Jr., M.D.**

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

**REPORT TITLE: STUDIES OF NEUROENDOCRINE ABNORMALITIES
IN BURN INJURY - CORTISOL AND CORTICOTROPHIN
IN BURNED SOLDIERS**

**US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234**

Period covered in this report: 1 October 1980 - 30 September 1981

**Investigators: George M. Vaughan, M.D., Major, Medical Corps
Richard A. Becker, M.D.
John P. Allen, M.D.
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Arthur D. Mason, Jr., M.D.**

Reports Control Symbol MEDDH-288(R1)

Based on sequential early morning samples, plasma cortisol concentration was elevated in proportion to burn size. Plasma corticotrophin (ACTH) was not correlated with burn size, suggesting that factors other than ACTH contribute to the elevated cortisol. Cortisol levels did not fall on the days prior to death in nonsurvivors. During 24-h sampling, burn patients exhibited a fitted cortisol curve mean that was elevated in proportion to burn size, a rhythm amplitude that was significantly less than that in uninjured controls, and a normal peak time. Metabolic rate, rectal temperature and urinary catecholamine excretion were also elevated in proportion to burn size. Though plasma cortisol was positively correlated with metabolic rate and with temperature, this appeared to result from a common relationship of these variables with burn size. On the other hand, urinary catecholamine values significantly reduced the residual variance of metabolic rate and temperature after accounting for variance related to burn size. Cortisol appears to be less prominent than catecholamines as a possible mediator of the elevated thermogenesis.

**Cortisol
Corticotrophin**

STUDIES OF NEUROENDOCRINE ABNORMALITIES IN BURN INJURY - CORTISOL AND CORTICOTROPHIN IN BURNED SOLDIERS

Burn injury results in hypermetabolism that has been thought to result from elevated catecholamine secretion (1). However, enhanced adrenocortical secretion after burns has also been shown by measurements of urinary corticoid excretion (2) and plasma cortisol concentration (3, 4, 5, 6). Such a response is expected as an immediate result of stress and is reflected in the rise of plasma cortisol within eight hours of various types of injury (7). However, like the ensuing hypermetabolism, the adrenocortical response to burn injury extends well past the immediate post-traumatic period (8) and thus represents another feature of the altered internal milieu that characterizes the body's reaction to severe injury. Furthermore, it has recently been proposed that cortisol is the major hormonal determinant of the postburn metabolic response (9).

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Plasma corticotrophin (ACTH) concentration varies widely but is often normal after major burns (10). Though simultaneous cortisol levels were not reported, this suggests a lack of correlation between ACTH levels and expected excess cortisol secretion after burns. In addition, previous studies in subjects without burn injury (11, 12, 13, 14, 15) have suggested that plasma cortisol rises in response to a rise in core temperature. In order to assess the relationships of plasma cortisol to ACTH, core temperature, metabolic rate, urinary catecholamine excretion, and severity of injury, we have used multiple regression analysis to evaluate these variables measured in the same patients during their postburn course.

PATIENTS AND METHODS

Thirty-six men, aged 17-23 years and burned in a single accidental gasoline fire, were air transported for treatment 122⁰ longitude to the East from the site where they had been stationed and injured. They were admitted to this burn center on the second postburn day (PBD). Because of the suppression of thyroid hormone levels that occurs after burn injury, particularly in deteriorating patients (16), they were entered into a study to evaluate the effect

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of triiodothyronine (T_3) administration. Eight of these patients had small burns over 2-7.5% of body surface and were designated as controls (CONT), since this degree of injury is not associated with measurable alteration in metabolic rate (1). The remaining 28 had a total burn size (TBS) of 18-93% of body surface and were randomly assigned in double blind fashion to treatment with either a placebo or T_3 , 200 μ g per day orally or by nasogastric tube, until their wounds were healed. This dose had previously been found to normalize T_3 levels in burn patients. Because four patients died while receiving placebo (NSURV) and four while receiving T_3 treatment (NSURV-TX), the data were analyzed within the five groups characterized in Table 1.

Beginning on PBD 3-5, and then approximately thrice weekly between 0500 h and 0700 h when the patients were under resting conditions in the supine position, blood was collected in plastic syringes, placed into heparinized plastic tubes on ice and centrifuged at 4°C. The plasma was then frozen at -70°C. Cortisol (supplies from Clinical Assays, Boston, Massachusetts) and corticotrophin (ACTH) (17) were measured by radioimmunoassay. Results of plasma thyroid hormone and catecholamine studies will be presented elsewhere. On the days of blood sampling, 24-h urine collections were obtained for measurement of creatinine and total catecholamines (18). The rectal temperature on the morning of sampling was recorded. Clinical sepsis (obtundation or ileus) was recorded if present at the time of sampling. At weekly intervals, following at least an eight-hour period free of caloric intake, resting metabolic rate (MR) based on O_2 consumption (19) was measured in all surviving patients.

Data analysis was focused on the PBD 3-26 period, because a major decrement in catecholamines and MR occurred by PBD 26, the control patients were available for varying periods up to this time, and all survivors were sampled throughout this time (Table 1). All samples obtained within 24 h of dopamine or glucocorticoid administration were discarded from analysis. In one assessment of the data, each variable was considered as the mean value of all measurements of that variable obtained during the PBD 3-26 period for each patient, but since major changes in most variables took place over this time, the time factor was accounted for in separate analyses using individual values of variables in a standard stepwise multiple linear regression program (BMDP, UCLA) performed on a PDP 1140 computer. To assess curvilinear dependent variability, independent variables TBS and PBD were also entered as TBS^2 and PBD^2 into the multiple regression analyses.

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TABLE 1.

	N	%TBS (mean)	%TBS (range)	Final Sample (PBD)
CONT	8	4.5	2 - 7.5	5 - 26
SURV	10	44.3	18 - 82	33 - 38
NSURV	4	68.4	55 - 93	5 - 54
SURV-TX	10	45.3	28 - 75	31 - 73
NSURV-TX	4	72.9	62 - 85	12 - 19

TBS, total burn size as % body surface; PBD, postburn day; CONT, controls with small burns; SURV, placebo-treated survivors; NSURV, placebo-treated nonsurvivors; SURV-TX, T_3 -treated survivors; NSURV-TX, T_3 -treated nonsurvivors.

Hormonal variables were added to these to determine if they would significantly ($p < 0.05$) reduce the residual dependent variances. To assess the impact of T_3 treatment or clinical sepsis, one of these was included as an independent variable by assigning its presence a value of 1 and its absence a value of 0.

In a separate study, nine normal subjects aged 24-36 years (seven men and two women) and eight burn patients aged 19-60 years (six men and two women) were sampled every two hours through an indwelling venous catheter over a 24-h period for serum cortisol determination. The results were evaluated by individual cosinor curve fitting, as well as by comparison of estimates of group cosinor parameters (20).

20. Batschelet E: Statistical rhythm evaluation. In Ferin M, Franz H, Richart RM, and Vande Wiele R (Eds) *Biorhythms and Human Reproduction*. New York: John Wiley & Sons, 1974, pp 25-35.

RESULTS

The more severely injured patients had higher cortisol concentrations than did CONT (Figure 1). Multiple regression analysis showed no effect of T_3 treatment or the presence of clinical sepsis on cortisol beyond the variation accountable by TBS and PBD. Though cortisol was not related to ACTH in the T_3 untreated patients over PBD 3-26, consideration of all groups did allow inclusion of ACTH as a predictor of cortisol (Table 2). The positive relationship between cortisol and TBS seen during this interval was beginning to develop by PBD 3 and PBD 5 (Figure 2). The relationship of ACTH to cortisol was not discernable using mean values (Figure 1), and ACTH was unrelated to TBS or PBD on multiple regression analyses. Cortisol was not correlated with days before death in nonsurviving patients. The final samples were taken within 48 h of death in six of eight nonsurvivors and within 24 h in three.

Multiple regression analyses did not demonstrate a significant effect of treatment with T_3 on MR, rectal temperature or urinary catecholamine excretion. During the interval of PBD 3-26, MR was related to TBS and PBD (Figure 3). Though cortisol was positively related to MR and to temperature, and both were related to TBS (figures 3 and 4), multiple regression analyses including all groups indicated that MR and temperature were not significantly correlated with cortisol nor with each other beyond their individual relationships to TBS and PBD. Excretion of total urinary catecholamine per gram of creatinine (UCA) was elevated in proportion to TBS, and MR and temperature were correlated with UCA (Figure 5). Multiple regression analyses showed that after correlation with TBS and PBD, the residual variance of both MR and temperature was significantly ($p < 0.001$) reduced by including values of UCA. TBS and PBD accounted for 70-75% of the variation in MR, and UCA accounted for an additional 5-9% (Table 2). Cortisol was not correlated with UCA beyond their individual relationships to TBS and PBD.

The cortisol rhythm mesor (fitted curve mean) was significantly elevated above normal in burn patients ($p < 0.01$), and within the burn group, the cortisol mesor was positively correlated with TBS ($p < 0.05$). The burn group rhythm was statistically significant (Figure 6), and group rhythmicity was still evident ($p < 0.05$) after excluding the two patients with the smallest burns (TBS 5.5 and 13.8%; remaining TBS 36-67%). That cortisol rhythmicity was reduced in the burn patients is indicated by absence of a statistically detectable rhythm in all individual patients (but present in eight of nine normal individuals) and a group amplitude-acrophase point significantly ($p < 0.05$) different from normal with an amplitude reduced by one-half. The timing of the rhythm appeared normal in the patients.

DISCUSSION

Cortisol concentrations (Figure 1) in the patients with very small burns were lower than usual morning normal values (8-25 $\mu\text{g/dl}$) possibly because the morning samples taken in this study would have represented an 8-h earlier (evening) time in their pre-injury location. In the new location and before possible rhythm re-entrainment, which was not identified by nyctohemeral sampling, in this population, cortisol would be expected to be near the nadir of its daily cycle at the time of sampling.

The single time point morning samples did allow observation of elevated plasma cortisol in proportion to burn size in the first four weeks after injury in adults. This confirms a similar relationship reported in a study of both children and adults during the first two weeks postburn (6) and in children during the first four weeks (5). In addition, we found cortisol concentration to be related to burn size as early as PBD 3 or 5. This elevation of plasma cortisol concentration is reflected in elevated values for the entire 24-h period in proportion to burn size. Molteni et al (21) found no rhythmicity in cortisol during the first four postburn days, with a trend toward the normal rhythm apparent by the fifth day. Rhythmicity was present at the time our nyctohemeral samples were taken (PBD 7-29). The suppression of rhythm amplitude that we observed may represent another expression of injury-induced alteration of hypothalamic function, which also includes elevated core temperature, an elevated ambient temperature of optimal comfort, suppressed growth hormone response to hypoglycemia (22) and excess sympathetic tone and hypermetabolism (1).

21. Molteni A, Warpeha RL, Brizio-Molteni L, Alvertson DF, and Kaur R: Circadian rhythms of serum aldosterone, cortisol and plasma renin activity in burn injuries. *Ann Clin Lab Sci* 9:518-523, 1979.

22. Wilmore DW, Orcutt TW, Mason AD, Jr., and Pruitt BA, Jr.: Alterations in hypothalamic function following thermal injury. *J Trauma* 15:697-703, 1975.

A recent report (23) suggests that reduced urinary excretion of cortisol plus cortisone is associated with mortality. Such data are difficult to interpret, because it has been suggested that conversion of cortisol to cortisone is positively influenced by body temperature and negatively by elevated cortisol secretion (11), and the relative hepatic and renal extractions of these compounds in traumatized patients are not known. We found no fall in plasma cortisol associated with death, in agreement with similar findings in other studies of urinary corticoid excretion and plasma cortisol levels (21). Cortisol is reportedly elevated in bacterial sepsis (24). However, in our injured patients with already elevated cortisol, we did not see further elevation of cortisol levels during clinical sepsis.

The physiological significance of elevated cortisol might be diminished if there were a sufficient elevation in plasma binding to prevent an elevation of free cortisol in burn patients. However, this appears not to be the case, since Mortensen et al (4) found a decrease, roughly proportional to burn size, in cortisol binding by human postburn sera. More dramatically, Wise et al (6) showed that the decreased serum binding capacity in burn patients was associated with a two- to sevenfold rise in the free portion of total serum cortisol compared to that in normal volunteers. Thus the vigorous postburn response is even more robust for free than for total cortisol levels.

Although Dolecek et al (10) found a rough correlation of ACTH with burn size, they state that quite normal or only slightly elevated ACTH levels were found in some patients after major burns and that ACTH levels were unpredictable and exhibited a wide range of values. Our results are similar, except that we also found no correlation of ACTH with burn size or time after injury. Using mean values over PBD 3-26, cortisol also was not significantly correlated with ACTH (Figure 1), though mean cortisol was closely correlated with burn size (Figure 2). Such observations suggest that factors other than plasma ACTH concentration are at least partially responsible for the large postburn cortisol response. Excessive adrenal responsiveness to ACTH is unlikely, in that Bane et al (25) found no response of plasma cortisol after ACTH injection in either of two burn patients tested. The afferent portion of the adrenocortical

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25. Bane JW, McCAA RE, McCAA DS, Read VH, Turney WH, and Turner MD: The pattern of aldosterone and cortisone blood levels in thermal burn patients. *J Trauma* 14: 605-611, 1974.

response to injury appears to be neurally mediated (26). Possible efferent control of adrenocortical function that does not involve ACTH is also suggested by previous reports. Spinal cord section blocks circadian rhythmicity of corticosterone in rats (27), normal human subjects exhibit nocturnal pulses of cortisol that do not appear associated with pulses of ACTH (28) and hemorrhage in dogs provokes a rise in plasma cortisol without a preceding rise in ACTH (29). However, ACTH may play some role, perhaps a permissive one, in maintaining cortisol levels in burn patients. In analyses with larger numbers of samples, unrestricted according to group, concentration of ACTH was found to be related to that of cortisol (Table 2).

Metabolic rate (Figure 3) and body core temperature (Figure 4) are both elevated as a function of burn size. This has already been reported for metabolic rate (1). These relationships support the thesis that the elevated thermogenesis in burn patients results not from attempts to restore heat content lost excessively to the environment ("externally cold") but rather from an altered setting of neural centers that results in heat production sufficient to raise core temperature to a higher level ("internally warm") (1). The correlations of cortisol with temperature and metabolic rate (Figure 4) could suggest either an interaction between cortisol and thermogenesis or independent responses of cortisol and thermogenesis to severe injury. The former possibility should be considered, because in normal human subjects, induced hypothermia lowers plasma cortisol (14), and hyperthermia raises it (11). During swimming at various water temperatures, serum cortisol rose only in experiments in which body temperature increased (13). In addition, hypothermic patients had reduced responsiveness to injected ACTH (12), and elevated serum cortisol paralleled the degree of hyperthermia in several febrile illnesses (15). The interaction between temperature and cortisol seems likely for the direction of an influence of temperature on cortisol levels, and it is thus possible that hyperthermia may account at least in part for the elevated cortisol in burn patients.

26. Wilmore DW, Long JM, Mason AD, Jr., and Pruitt BA, Jr.: Stress in surgical patients as a neurophysiologic reflex response. *Surg Gynecol Obstet* 142:257-269, 1976.

27. Allen-Rowlands CF, Allen JP: Spinal cord section blocks circadian rhythmicity of corticosterone in the rat. *Fed Proc* 34:43 (Abstract 1148), 1973.

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29. Gann DS: Cortisol secretion after hemorrhage: Multiple mechanisms. *Nephron* 23:119-124, 1979.

The function of glucocorticoids in thermogenesis of experimental animals has been reviewed by Deavers and Musacchia (30). The results show that whereas glucocorticoid is necessary for animals to restore body temperature following induced hypothermia, there is no effect of glucocorticoid on thermogenesis in animals not exposed to cold. However, in burn patients, thermogenesis is reset around a higher core temperature, allowing the possibility that usual environmental temperatures may be sensed as cold (1). In this condition, cortisol might be envisioned as a potential mediator of hypermetabolism. Plasma cortisol has been linked to some of the metabolic substrates or intermediates in burn patients, which are thought to be related to hypermetabolism. Volenec et al (31) found cortisol correlated with plasma glucose level. Alberti et al (8) noted correlations of cortisol with alanine, lactate, free fatty acid, ketone and urea levels in burn patients. By raising cortisol in normal subjects using ACTH injections, they produced a rise in circulating glucose, lactate and alanine. Those authors concluded that cortisol plays the major role in the catabolic response to injury.

However, there are several limits on interpretation of those data (9). Although in that study the known gluconeogenic and catabolic nature of cortisol was evident in the normal subjects, the relationship of this hormone to the metabolic response of burn patients was not assessed in a manner which would establish that cortisol bore a better relationship to metabolic response than did extent of injury or determine whether variation of metabolic response was more closely related to variation in other gluconeogenic hormones after accounting for the extent of injury. The authors mentioned that plasma glucagon levels and excretion of metanephrine were elevated. Their results were essentially limited to the first five postburn days. Finally, the metabolic variables reported are elements of biochemical pathways in energy metabolism and are indirect and restricted reflections of the elevated resting metabolic rate that occurs after burn injury. Although we have found that cortisol level was closely correlated with both metabolic rate and temperature, this appears to reflect the general relationship of metabolic rate and temperature to burn size and postburn day, and no specific connection between cortisol and thermogenesis was identified. That cortisol may contribute to hypermetabolism is not excluded by these results. However, consideration of urinary catecholamine excretion reduced the residual variance of both metabolic rate and temperature after accounting for variation associated with burn size and time since injury. These findings corroborate a role of catecholamines and suggest less importance of cortisol in postburn hypermetabolism.

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TABLE 2.

Variables, intercept and coefficients	Including T ₃ -treated	Samples n	r ²
Cortisol = 8.6 + 0.31 TBS	-	148	0.39
Cortisol = -10.1 + 0.32 TBS + 2.7 PBD - 0.1 PBD ² + 0.05 ACTH	+	264	0.47
MR* = 42.4 + 0.44 TBS - 0.68 PBD	-	38	0.70
MR = 38.1 + 0.33 TBS - 0.59 PBD + 0.062 UCA	-	38	0.79
MR* = 44.4 + 0.495 TBS - 0.853 PBD	+	64	0.75
MR = 39.3 + 0.42 TBS - 0.71 PBD + 0.05 UCA	+	64	0.80

*UCA values omitted from regression; TBS, total burn size; PBD, postburn day; MR, resting metabolic rate; UCA, urinary catecholamine

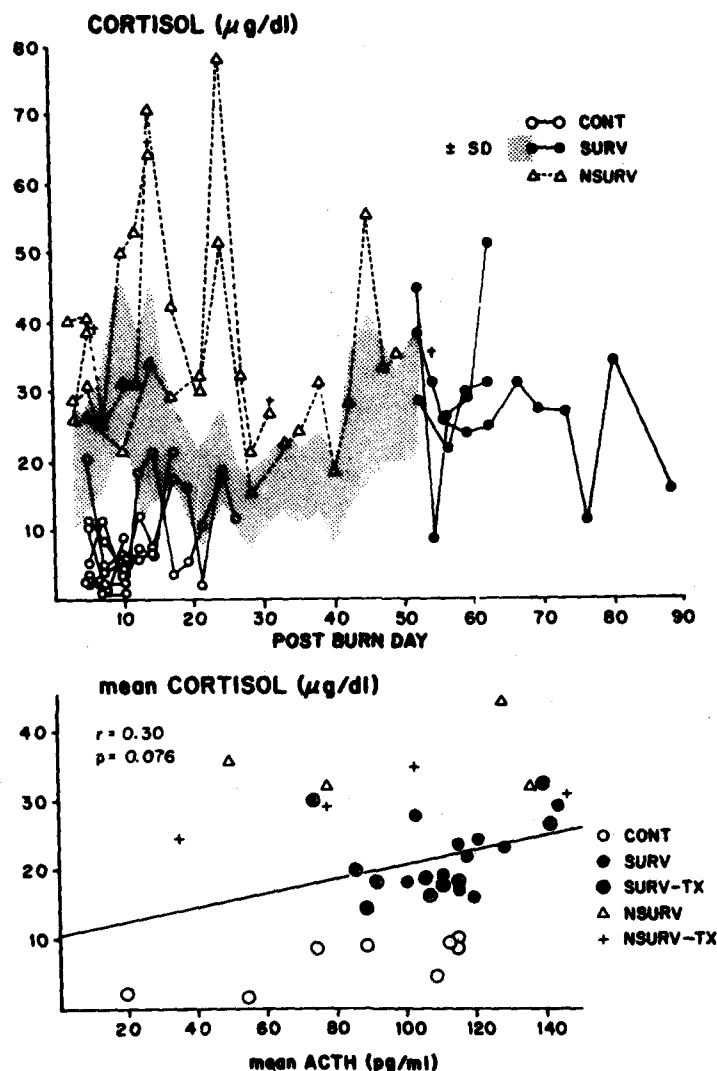


Figure 1. Plasma cortisol concentration samples serially on mornings following burn injury. The crosses nearest a final symbol for nonsurvivors indicate the day of death (top panel). Linear regression of mean cortisol and ACTH as the mean taken over postburn days 3-26 for each patient (bottom panel). For group designations in this and subsequent figures, see Table 1.

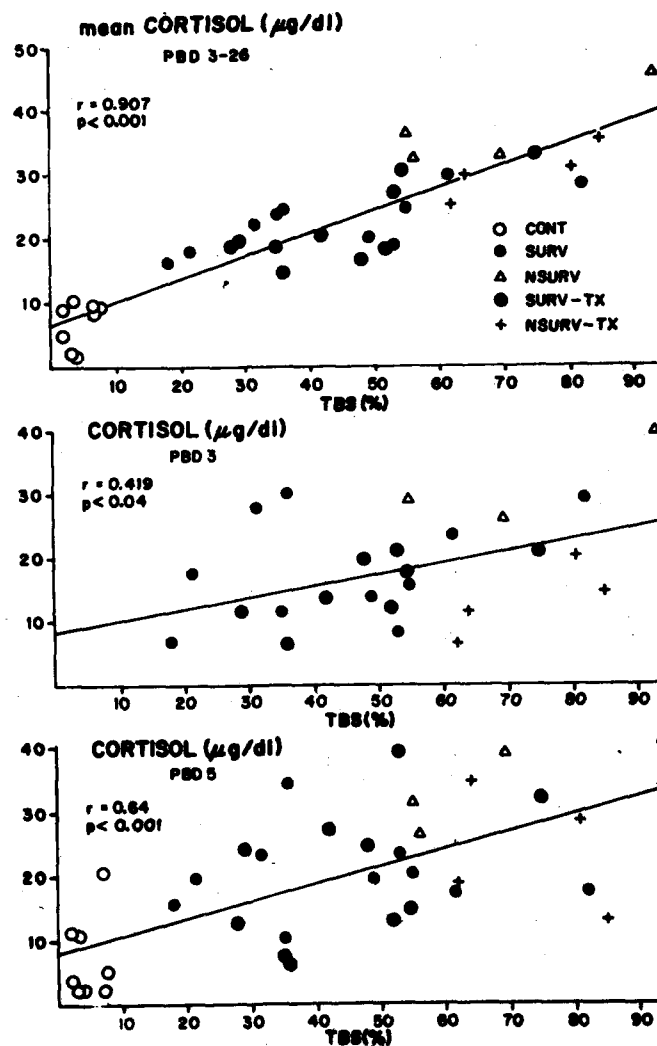


Figure 2. Linear regressions of plasma cortisol and total burn size (TBS) based on mean values over postburn day (PBD) 3-26, or values for PBD 3 or PBD 5.

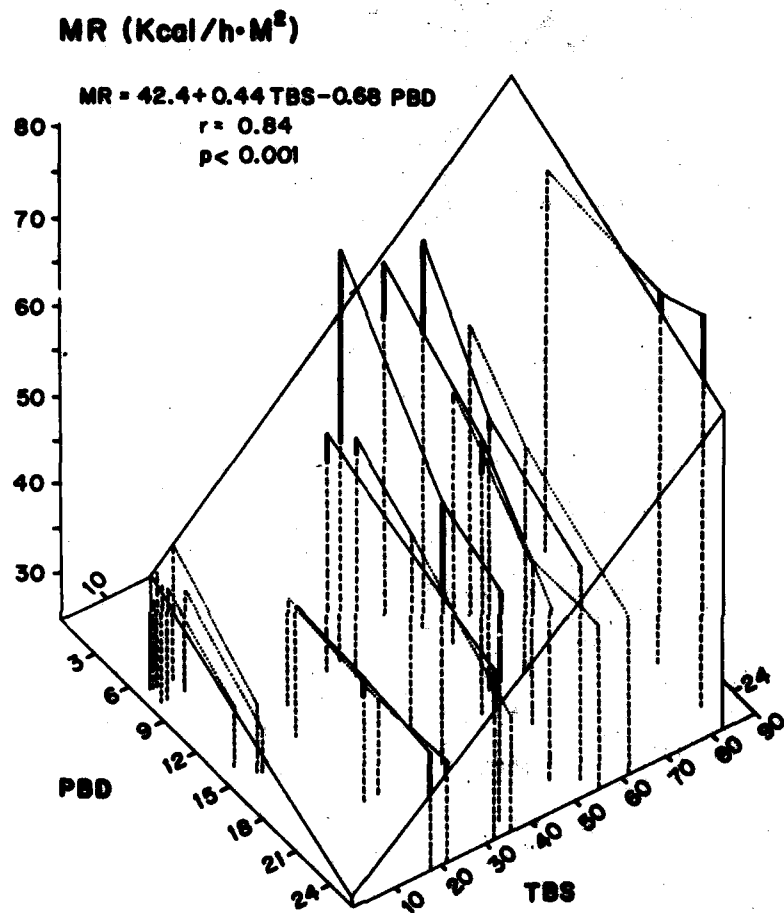


Figure 3. Relationship of metabolic rate (MR) to total burn size (TBS) and postburn day (PBD) for patients not treated with triiodothyronine (T₃). The equation for the plane of best fit is indicated.

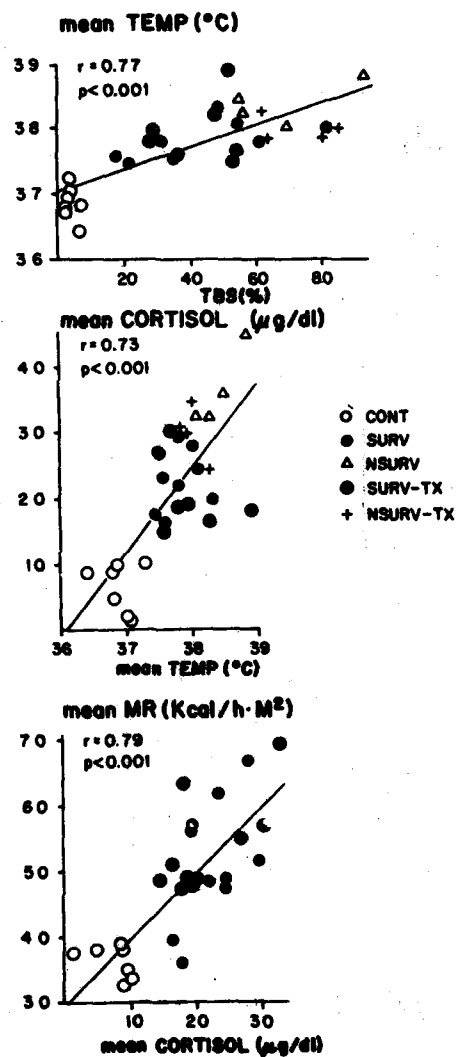


Figure 4. Linear regressions between rectal temperature (TEMP) and total burn size (TBS), and between TEMP or metabolic rate (MR) and plasma cortisol, based on mean values for each patient over postburn days 3-26.

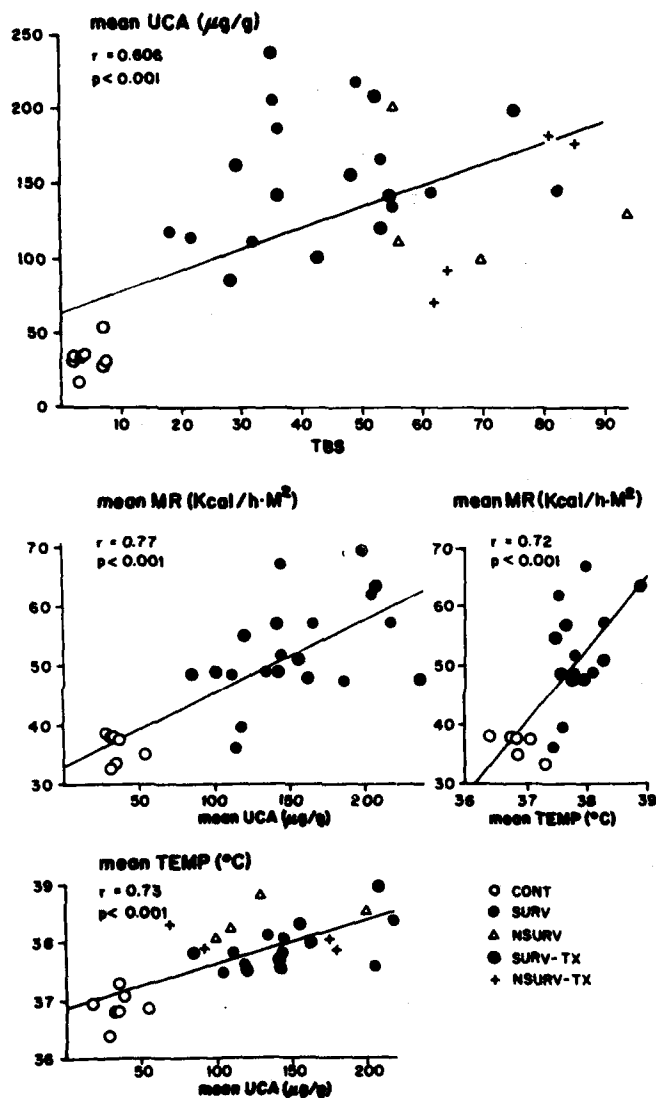


Figure 5. Linear regressions between excretion of total urinary catecholamines (UCA, $\mu\text{g/g}$ creatinine) and total burn size (TBS), metabolic rate (MR), or rectal temperature (TEMP), and between MR and TEMP, based on the mean values for each patient over postburn days 3-26.

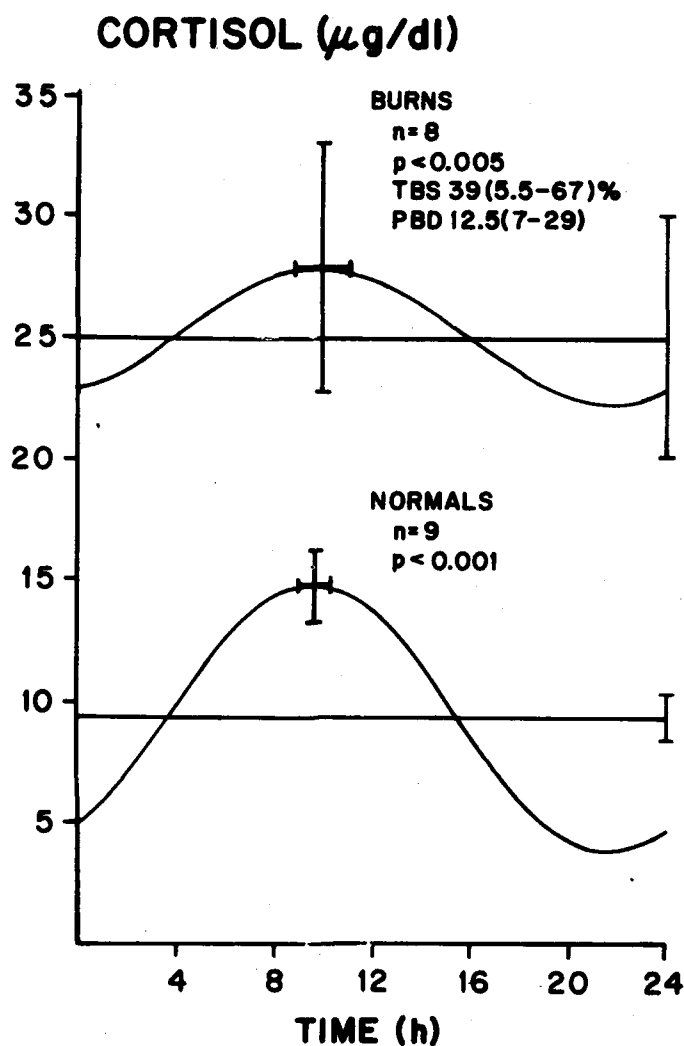


Figure 6. Best-fit group cosinor curves for plasma cortisol based on samples taken at 2-h intervals for a 24-h period and their individual best-fit curves. The error bars are SEM for curve peaks (mesor plus amplitude) and peak times (acrophases), and for curve means (horizontal lines). TBS, total burn size. PBD, postburn day.

PUBLICATIONS/PRESENTATIONS

Annual Meeting of American Association for the Surgery of Trauma,
Hot Springs, Virginia, 17 September 1981.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

**PROJECT TITLE: STUDIES OF NEUROENDOCRINE ABNORMALITIES
IN BURN INJURY - HUMAN MELATONIN AND CORTISOL
DURING ACUTE STRESS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
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1 October 1980 - 30 September 1981

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Reports Control Symbol MEDDH-288 (R1)

Unclassified

ABSTRACT

PROJECT NO. 3S162772A874-00, APPLIED RESEARCH

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The stresses of insulin hypoglycemia, pneumoencephalography, and exercise were not associated with a rise in human plasma melatonin, although cortisol or other hormonal markers of stress did increase. Patients with lesions in the neural pathway, described as controlling the pineal melatonin rhythm in animals, had a suppressed plasma melatonin rhythm similar to the depressed cortisol rhythm seen in other patients with lesions of the pituitary-adrenal axis. In relation to the melatonin and cortisol rhythms, the presence of one does not require that of the other. However, when both are present, they appear linked in time with a phase separation of 4.4-9.3 h. In visually and neurologically intact humans, the melatonin rhythm may represent the output of a stable oscillator with a signal entrained to the light-dark cycle and relatively free from acute perturbation by stress or changes in other hormones. Such a marker for the human neural biological clock may have applications in research on rhythms in burn patients.

Melatonin
Cortisol

STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN BURN INJURY - HUMAN MELATONIN AND CORTISOL DURING ACUTE STRESS

Post-Cartesian thinking decreed that the pineal gland was not only the seat of the soul but also the seat of mental health (1). Even in modern times, pineal extracts have been administered to patients with hypersexuality (2, 3) and schizophrenia (4), with at least a statistically beneficial effect in the latter case. However, most of the more familiar developments in modern pinealology center around the pineal's ability to respond to light deprivation and thereby to suppress the reproductive system in experimental animals (5, 6). What little endocrinological information that has been obtained in people with altered light perception or bearing pineal tumors suggests that the human pineal could also function in the same way (6-9).

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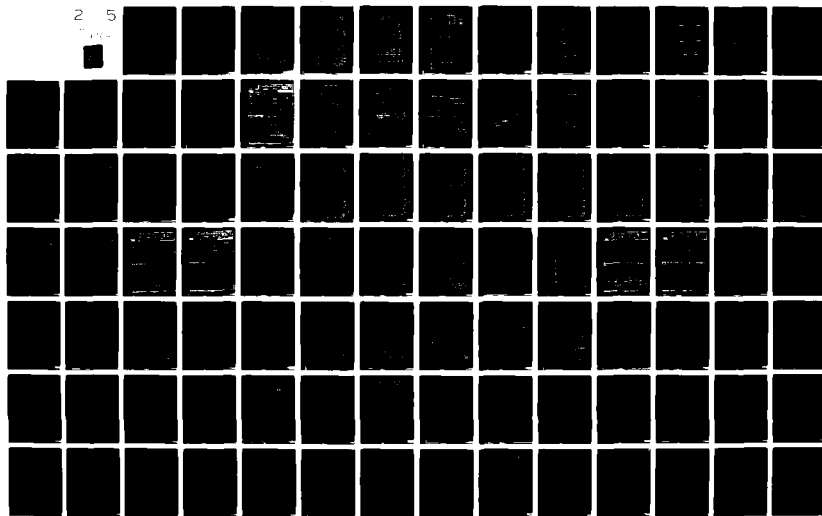
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Synchronous with these developments, there have been: (1) identification of melatonin, the pineal factor that lightens tadpole skin (10); (2) investigation of the nocturnal surge in pineal melatonin synthesis, which occurs in a rhythm entrained to the light-dark cycle (11); (3) observation of a similar rhythm in humans with a nocturnal melatonin surge in pineal (12), plasma (13, 14) and urine (15); and (4) the determination that properly timed (evening) daily melatonin injections can cause reproductive collapse despite a long photoperiod in hamsters (16, 17), mimicking the action of the pineal stimulated by light deprivation. So far, there seems to be little evidence for an acute effect of melatonin on the human reproductive system (18, 19).

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Melatonin has received some attention as a possible psychoactive hormone (20). Though exogenous melatonin can produce sedation (21) or sleep (22) with vivid dreaming (23), these findings have not been universal (18). The major surge of endogenous melatonin occurs during the usual sleep period, but daytime sleep occurs in the absence of a surge (24) until after a few days of sleep-wake phase reversal when the melatonin rhythm begins to adjust (25). Melatonin does not appear to be a major physiologic mediator of sleep.

One of the more intriguing features of melatonin in experimental animals is that the nocturnal surge in melatonin synthesis depends upon an intact neural pathway from the anterior hypothalamus through the brain stem and upper spinal cord, thence out through the sympathetic system innervating pineal cells through beta-noradrenergic neurotransmission (11, 26). Such a relationship to the sympathetic nervous system suggests that melatonin could be either a signal or a mediator of stress. In keeping with this notion, rat pineal melatonin synthesis is stimulated by insulin hypoglycemia (27), immobilization (28)

20. Wetterberg L: Melatonin in psychiatric conditions. In: Melatonin--Current Status and Perspectives. Birau N, and Schloot W (Eds.) Oxford, England: Pergamon Press, pp 365-370, 1981.

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or injection of L-dopa (29). In humans, propranolol inhibits the nocturnal rise in plasma melatonin (30). The nocturnal surge in urinary melatonin did not occur in patients with cervical spinal cord transection (31). Thus, it is likely that melatonin secretion has the same neurological basis in humans as in experimental animals.

The link between the sympathetic system and melatonin is perhaps paradoxical, since a rise of endogenous melatonin is temporally associated with sleep and administration of large amounts of this hormone may produce sedation and inhibition of seizure activity (23, 32, 33). In the subsequent parts of this communication, we will focus on the effects of acute stresses on plasma melatonin-like immunoactivity and on the neurologic basis for its rhythm in human subjects.

For the melatonin analyses described in this communication, the Rollag antibody (34) was employed. As previously described (35), compounds similar in structure to melatonin cause no significant binding inhibition, dilutions of plasma inhibit binding of tracer in a fashion parallel to the standard curve, adding different amounts of melatonin to plasma produces the expected binding inhibition, and immunoactivity in the plasma chloroform extract used in the assay co-migrates with ^3H -melatonin on thin layer chromatography. Washing the buffer-dissolved extracts of human plasma with petroleum ether did not remove melatonin-like immunoactivity in our system. Because workers using mass

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spectrometry have reported obtaining somewhat lower levels in plasma (36) than we usually find. It may be appropriate to consider our melatonin results as melatonin-like immunoactivity exhibiting a nyctohemeral pattern similar to that reported by others for melatonin. Melatonin, as measured with this antibody, is suppressed by nocturnal light in experimental animals (34), but not by normal intensity of room light applied at night in humans (37) in similarity with reports by others (25, 38), and it is not stimulated by daytime sleep periods (24) in similarity with results of others (39). Results of other hormones reported herein were obtained using standard radioimmunoassay.

RESULTS

Melatonin in Acute Stress and After a Dose of L-Dopa. Our results of plasma melatonin during stress or after a dose of L-dopa have been reported (24). A standard insulin tolerance test (ITT) was performed in three women and eight men undergoing examination for an abnormal appearance of the sella turcica on skull radiographs. Blood was sampled before and at 15-minute intervals for 75 minutes following insulin injection. Glucose concentration fell sufficiently to produce symptoms of sympathetic discharge in each subject. Following this, a significant rise in cortisol and growth hormone occurred; whereas, melatonin concentration did not rise.

Pneumoencephalography (PEG) was performed in the clinically indicated evaluation of 16 women and 7 men suspected of having pituitary disease. This procedure involves injection of air into the subarachnoid space and usually produces headache. In these cases, PEG took approximately 80 minutes, and blood was sampled at the beginning, near the middle and at the end. Mean growth hormone was elevated from the beginning (10 to 13 ng/ml throughout), and mean cortisol rose significantly from 18 to 27 at the middle and 31 g/dl at the end of the test, with no change in melatonin. In both the ITT and PEG, patients with normal baseline prolactin concentration exhibited significant rises in prolactin with no rise in melatonin.

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38. Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, and Markey SP: Light suppresses melatonin secretion in humans. *Science* 210:1267-1269, 1980.

39. Jimerson DC, Lynch HJ, Post RM, Wurtman RJ, and Bunney WE, Jr.: Urinary melatonin rhythms during sleep deprivation in depressed patients and normals. *Life Sciences* 20:1501-1508, 1977.

Two physically untrained young men each ran 100-yard sprints at three times over a 20-min period. Plasma was sampled at four times before and 12 times over the 14 hours following the first sprint. There was no discernable response in melatonin or cortisol, though growth hormone exhibited a dramatic surge. Tachycardia and sweating attested to a surge in sympathetic activity.

L-dopa, a catecholamine precursor, was administered to five normal volunteers in a single 500-mg oral dose. On another day, they received placebo in a single-blind fashion. Blood was sampled in each case at baseline, every 15 minutes for the first hour, every 30 minutes for the second hour, and at three hours following the dose or placebo. Although three subjects exhibited the characteristic rise in growth hormone peaking at 60 to 120 minutes, there was no consistent rise in melatonin.

Melatonin and Cortisol Rhythms in Patients With Neurological Lesions. Table 1 gives the characteristics of the subjects of this study and explains their grouping. They were all sampled on a research ward (lights off 2300 to 0700 hours) through an indwelling venous catheter, and all except one habitually slept at night. Both hormones were not measured in all subjects. Plasma hormone concentrations for each subject were sampled throughout one to two continuous 24-hour periods and fitted by computer using least squares cosinor regression to a curve with 24-hour periodicity (Fig. 3). Analysis of variance was used to assess presence of a rhythm in individuals, and substitution of the origin coordinates (zero amplitude) into the formula for the 95% confidence ellipse of the mean for the observed amplitude-acrophase pairs was used to assess a group rhythm (40). Melatonin values (without cosinor analysis) for patients 1, 6, 8 and 10 and for the HPL and PAL groups have been reported (24).

The group of controls included not only normal subjects, but also patients with illnesses not known to involve the pathway from the hypothalamus to the pineal. Inclusion of these patients in this group allowed for nonspecific variation due to illness, such that differences in the other groups may be viewed as more specific. The HPL group had lesions in the presumed hypothalamopineal pathway. Because three of these patients also had hypothalamic lesions, it was not surprising to observe altered pituitary-adrenal function in some of these patients. The patients in the group with pituitary-adrenal axis lesions (PAL) did not harbor lesions of the hypothalamopineal pathway.

40. Batschelet E: Statistical rhythm evaluation. In: *Biorhythms and Human Reproduction*. Ferin M, Halberg F, Richard RM, and Vande Wiele RL (Eds.). New York: Wiley & Sons, pp 25-35, 1974.

TABLE 1. Characteristics of Subjects for Rhythm Analysis

GROUP	SUBJ. NO.	AGE	MONTH	SAMPLING PERIOD (h)	MELATONIN n p*	CORTISOL n p*	COMMENT
CON (Control)	1						
	66	22	2	24	6 0.124	6 0.022	Mild depression
	27	27	2	48	19 < 0.001	19 < 0.001	
	6a		9	48	21 < 0.001	21 < 0.001	
	6b		9	48	20 < 0.001	20 < 0.001	Light occlusive blindfold
	6c		9	48	20 < 0.001	20 < 0.001	
	6d		9	48	20 < 0.001	20 < 0.001	
	6e		6	50	51 < 0.001	51 < 0.001	
	8	28	5	24	6 0.088		Leydig cell tumor
	10a	24	10	48	20 0.076	20 < 0.001	
	10b		10	48	20 0.020	20 < 0.001	Light occlusive blindfolds
	10c		10	48	21 0.243	21 0.005	
	13	23	11	48	61 < 0.001	59 < 0.001	
	14a	30	11	24	72 < 0.001	12 0.071	
	16a	60	2	24	9 0.035		Acromegaly, before operation
	16b		2	24	8 0.053		Hypertension, 10 mEq Na ⁺ diet
	17a	19	2	24	8 0.158		Hypertension, 250 mEq Na ⁺ diet
	17b		2	24	9 0.330		Hypertension, 10 mEq Na ⁺ diet
	18a	49	2	24	9 0.023		Hypertension, 250 mEq Na ⁺ diet
	18b		2	24	9 0.100		Hypertension, 10 mEq Na ⁺ diet
	19a	47	4	24	9 0.002		Hypertension, 250 mEq Na ⁺ diet
	19b		4	24	9 0.035		Hypertension, 10 mEq Na ⁺ diet
	23	22	5	27	22 < 0.001	49 < 0.001	Hypertension, 250 mEq Na ⁺ diet
	26	24	6	48	49 < 0.001	49 < 0.001	Sweating abnormality
	27	23	9	24	13 0.004	13 0.251	
	28	29	9	24	13 0.008	10 0.006	
	29	46	3	24		13 0.04	Alopecia areata
	30	59	3	24		13 0.021	Alopecia areata
	31	29	5	24		13 < 0.001	Alopecia areata
	32	18	5	24		13 0.115	Alopecia areata
	34	35	8	24		10 0.149	
	36	31	9	24		12 0.002	Healed burn injury

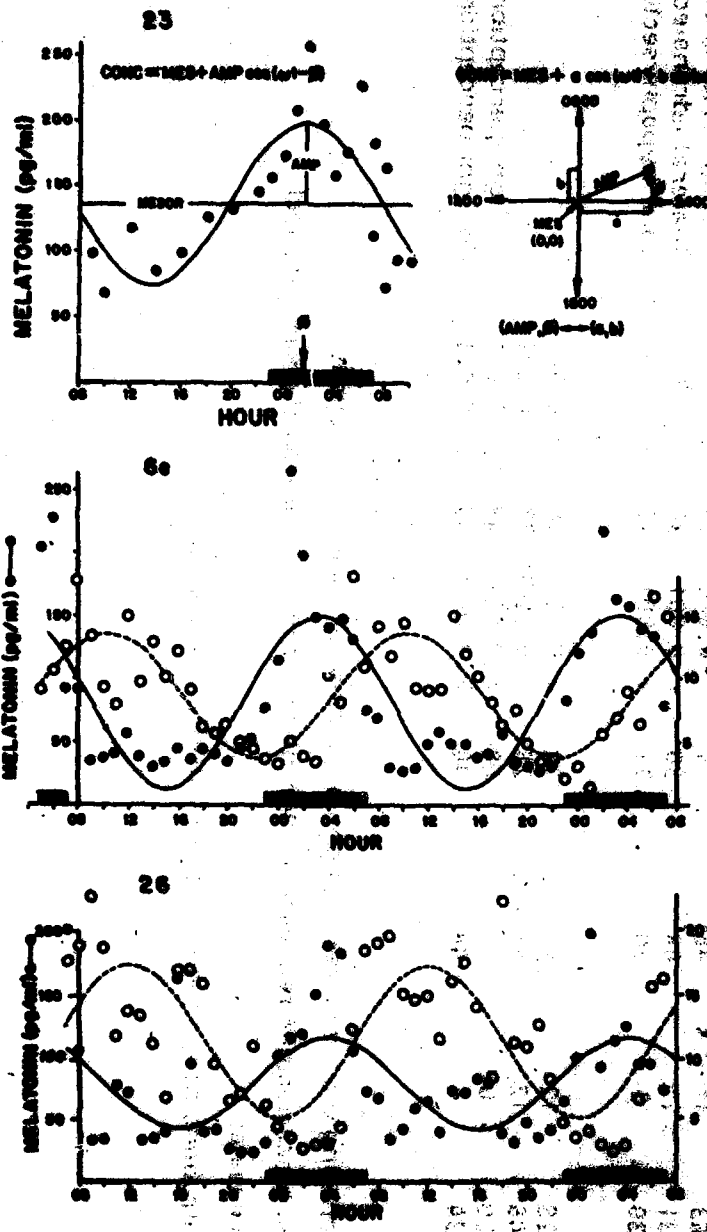


Figure 1. Curve-fitted hormone data in 3 control subjects. The amplitude (amp, $\frac{1}{2}$ curve excursion) and acrophase (ϕ , time of curve peak) can be expressed as polar (amp, ϕ) or rectangular (a, b) coordinates, and the mesor (mes) is the curve mean. In this figure and in Figure 2, the dark bar represents sleep time and darkness. In all the figures, the subject numbers are given and conform to the subject numbers in the table.

The blind subjects (BL) had retrolental fibroplasia since early life and what they considered as total blindness most of their lives. They were unable to detect discrete objects or a penlight. Two could discern the sudden onset of room lights from total darkness; the other two could not, and these were considered to have no light perception. They all reported a habit of normal sleep at night.

Subject 17 (not included in the analysis of the control group) was the only one who remained awake at night and slept during the afternoon. This was his customary daily routine. He was a normally sighted individual with idiopathic hypertension.

Figure 1 shows examples, using the conventional time-based display of hormonal data, representing control subjects. Figure 2 depicts examples of the HPL and PAL groups. The bottom panel represents the blind subject whose melatonin and cortisol acrophases were the most displaced. In Figure 3, each symbol represents the 24-hour mesor (best-fit curve mean) for a hormone cycle. Just as the PAL group had low cortisol mesors, so also the HPL group had low melatonin and no overlap with mesors of any other group. Two HPL patients (both with hypothalamic lesions) had cortisol mesors below control range. Blind patients had mesors for both hormones in the control range.

Figure 4 (upper panel) depicts the cosinor amplitude, acrophase (amp, ϕ) display for all the subjects having melatonin measurements. The broken lines represent the complete range of acrophase for all control subjects (mean acrophase 0229 hours). The points for PAL patients fall within the control range as do those for the two blind patients with minimal light perception. The points for the two blind patients with no light perception (particularly that for patient 25) fall outside control range, as do the points for the one subject (17) who has a reversed sleep-activity cycle. Patients with HPL all have small insignificant amplitudes near the origin, showing no overlap with amplitudes from any other group. As a group, their amplitudes show no preferred acrophase, whereas the control group ($p < 0.001$) and the PAL group ($p = 0.02$) each have significant mean amplitudes with a preferred acrophase. Because of the wide variation in BL acrophases, the group rhythm was not significant (no preferred acrophase, confidence ellipse overlaps the origin) despite highly significant individual rhythms.

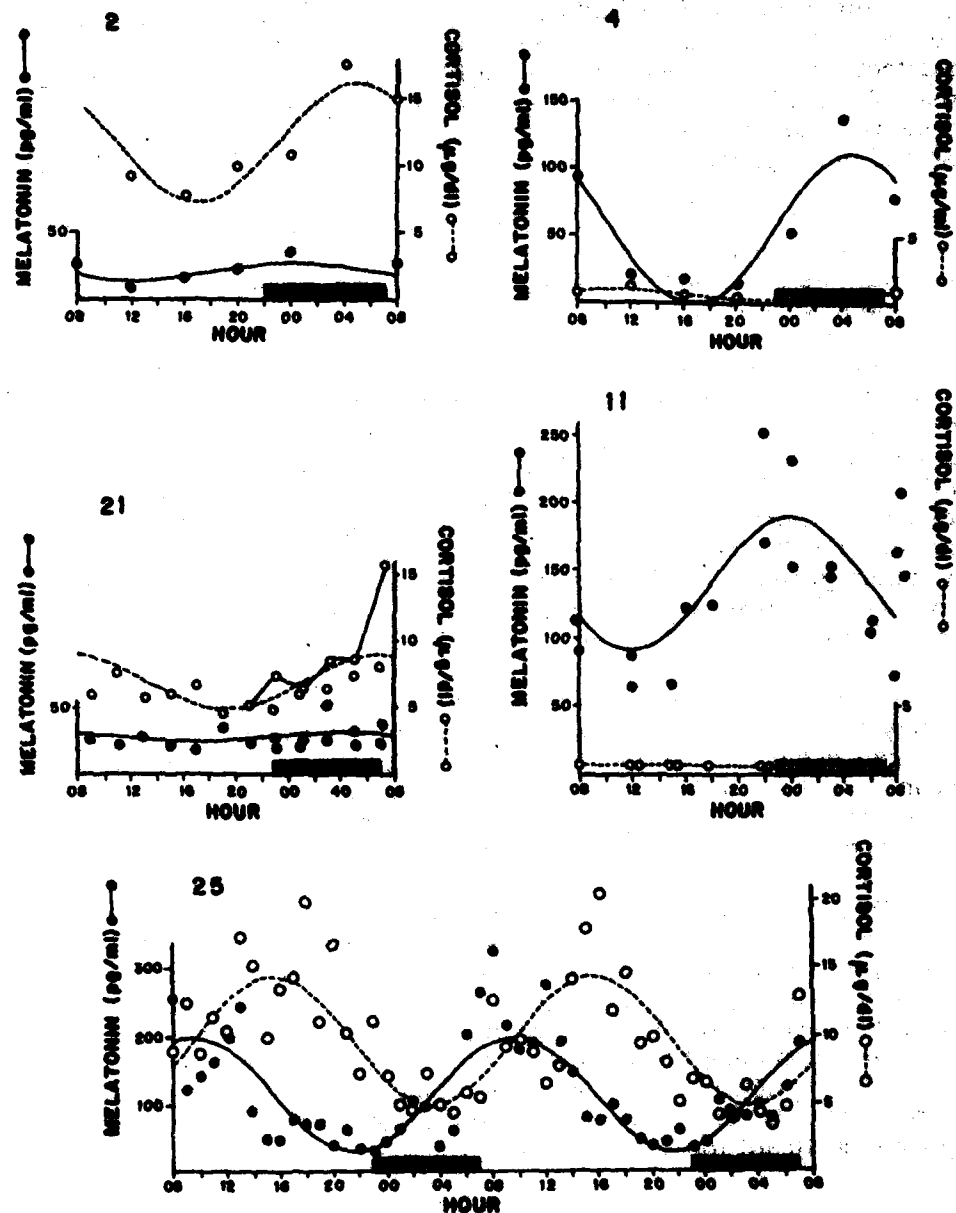


Figure 2. Examples of subjects from the HPL (2,21), PAL (4,11) and BL (25) groups. For subjects 21 and 11, data from two 24-h periods are superimposed. For subject 21, cortisol values from the night immediately preceding the other points are connected with a line.

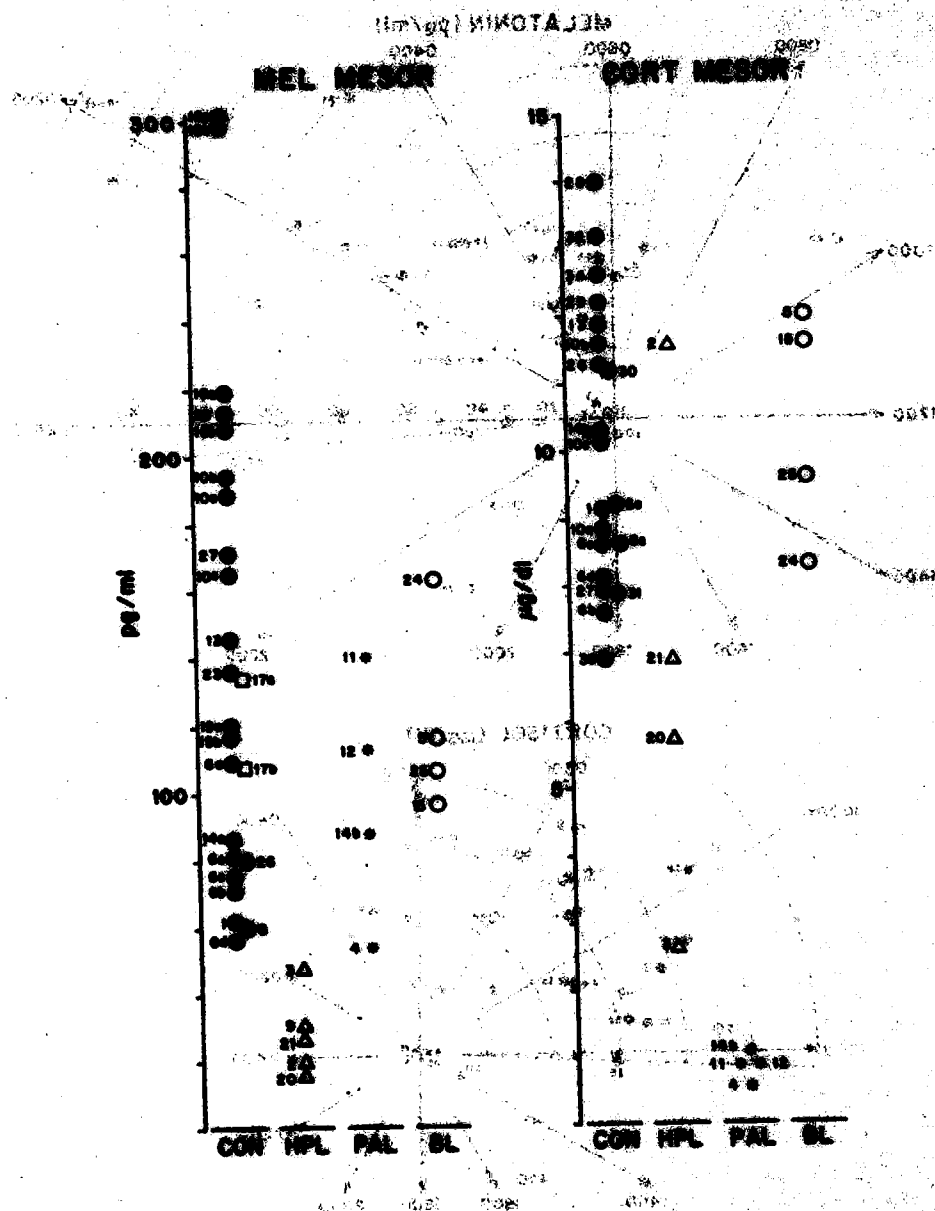


Figure 3. Mesors for melatonin (MEL) and cortisol (CORT) in controls (CON) and in subjects with a hypothalamic-pituitary-adrenal axis lesion (HPL), a pituitary-adrenal axis lesion (PAL) and a bilateral lesion (BL). Subject numbers are indicated.

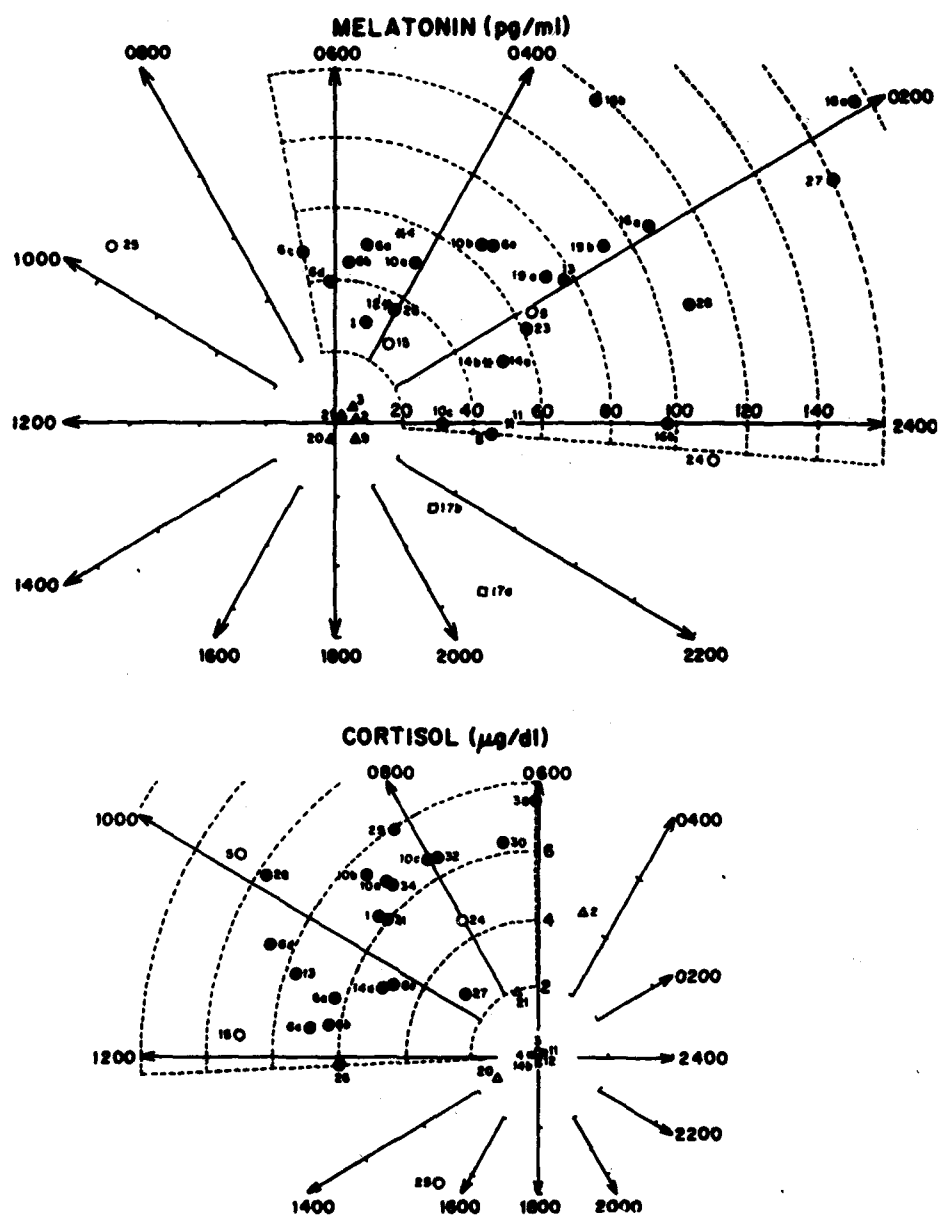


Figure 4. Amplitude, acrophase coordinates. The symbols, identified by subject number, are the same as in Figure 3. Amplitude (concentration units) is distance from the origin, and acrophase is counterclockwise angle expressed as time from midnight (2400).

Figure 4 (lower panel) depicts the cosinor display for the cortisol data. The complete acrophase range in controls is indicated by the broken lines (mean acrophase 0928 hours). The cortisol amplitudes of three HPL patients (each having a hypothalamic lesion) were below control range. Patient 20 was receiving cortisone replacement therapy, 25 mg orally, with breakfast and 12.5 mg after supper. One of the BL subjects with no light perception (25) had a cortisol acrophase outside the range for all other subjects. The patients with PAL (no glucocorticoid therapy) had small insignificant amplitudes near the origin. Group mean rhythm was significant only for controls ($p < 0.001$).

HPL patients all had lesions located in the pathway described as necessary for pineal melatonin synthesis in experimental animals. Patient 3 had a presumed germinoma of the pineal also ectopic in the hypothalamus, and patient 20 had hypothalamic destruction by a pituitary chromophobe adenoma. Patient 21 had a metastatic carcinoma in the hypothalamus. Patient 2 had progressive supranuclear palsy, a disease characterized by degeneration in the brainstem, a site through which the hypothalamopineal pathway must descend. Patient 9 was afflicted with Shy-Drager syndrome, characterized by pre-ganglionic sympathetic disease documented in this case by severe orthostatic hypotension together with normal basal plasma norepinephrine concentration that failed to rise in the upright position. Another patient (23) had a deficiency of sweating, but because of absence of orthostatic hypotension and a twofold rise in norepinephrine in the upright position (41), she was considered not to have a lesion of the noradrenergic sympathetic system and was placed in the control group. Her melatonin rhythm (Fig. 1) was significant. Interestingly, HPL patients had dramatic suppression of melatonin mesor (Fig. 3) and rhythmicity (Fig. 4) similar to the suppression of cortisol mesor and rhythmicity in patients with PAL. In view of the report of absent urinary melatonin rhythm in patients with cervical cord transection (31), it may now be appropriate to consider the possibility of a distinct syndrome characterized by a hypothalamopineal pathway lesion and suppressed melatonin rhythmicity. Such a concept suggests that a pathway similar to that in animals controls human melatonin secretion and that systemic melatonin in humans is derived largely from the pineal gland.

An attenuated pineal melatonin rhythm has been reported in very old hamsters (42). Though some HPL patients were above 60 years old, this was also true of a PAL patient and two controls. Furthermore, we

41. Kopin IJ, Lake RC, and Ziegler MG: Plasma levels of norepinephrine. *Ann Int Med* 88: 671-680, 1978.

42. Reiter RJ, Richardson BA, Johnson LY, Ferguson BN, and Dinh DT: Pineal melatonin rhythm: reduction in aging Syrian hamsters. *Science* 210: 1372-1373, 1980.

have observed apparently normal melatonin rhythms in parkinsonian patients in this age range (43). This suggests that the absent melatonin rhythm in HPL patients was not a reflection of their age.

Two patients with HPL (Fig. 2) showed a morning rise of cortisol to greater than 15 $\mu\text{g/dl}$, well within the normal limits (10 to 25 $\mu\text{g/dl}$) of the laboratory for morning cortisol. Patients with cervical cord lesions and without a nocturnal rise in melatonin (31) also had a normal cortisol rhythm. Since the melatonin rhythm can be present without a cortisol rhythm (figs. 2 and 4), the rhythm of neither hormone is driven by that of the other. However, melatonin and cortisol acrophase were correlated in control subjects for whom both hormones were measured, with ($p < 0.001$) or without ($p < 0.05$) inclusion of the BL group. Cortisol followed melatonin by 4.4 to 9.3 hours in controls and by 5.8 to 8.4 hours in BL. Therefore, it is likely that both rhythms when present are linked in time, which suggests a common central control mechanism. This is not unexpected, since the glucocorticoid and melatonin rhythms in rats are both controlled by the suprachiasmatic nucleus of the hypothalamus (44, 45). An unusual melatonin acrophase in the sighted subject who slept only during the day conforms to previous findings of dependency of the melatonin rhythm on the sleep-wake cycle (25). For two completely blind subjects, habitually sleeping at night, acrophase outside control range for melatonin (in both) and for cortisol (in one), as well as absent BL group hormone rhythms despite significant individual rhythms, suggest that these rhythms depend partly on the lighting cycle, as has been suggested

43. Vaughan GM, Bell RD, and Boyar RM: Melatonin rhythm in parkinsonism treated with a dopamine agonist. In: Pineal Function. Matthews CD, and Seamark RF (Eds.). Amsterdam: Elsevier/North-Holland Biomedical Press B.V., Chap. 4, pp 19-25, 1981.

44. Moore RY, and Klein DC: Visual pathways and the central neural control of a circadian rhythm in pineal serotonin N-acetyltransferase activity. Brain Res 71:17-33, 1974.

45. Moore, RY, and Eichler VB: Loss of circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res 42:201-206, 1972.

for cortisol (46, 47, 48). Whether normal timing of the rhythms in some blind subjects was a result of chance occurrence in a population with free-running rhythms, partial dependence of the rhythms on other environmental cues or slight residual light perception is not known.

CONCLUSION

The human melatonin rhythm appears to be dependent on the sympathetic nervous system, which system is usually thought to be most active during stress. Paradoxically, the usual sleep time is associated with the high point in circulating melatonin concentration. Our finding of lack of melatonin response during acute stress is in agreement with the results of others (49) and indicates that human melatonin secretion is controlled separately from several elements associated with the acute stress response. Relative independence of melatonin and cortisol levels in our study is in agreement with a reported inducible dissociation of melatonin and corticosterone patterns in rats (50). Also in agreement with that report, human melatonin appears more resistant to acute environmental influences and may thus represent a more stable marker for the biological clock. However, our results suggest a possible synchronization of the rhythms of melatonin and cortisol with each other and the light-dark cycle by a central mechanism. The neural pathway controlling the human melatonin rhythm may be similar to that in experimental animals. Burn patients have large elevations of plasma cortisol (Vaughan and colleagues, in preparation) and plasma catecholamines (Becker and colleagues, in preparation) on a chronic basis, and catecholamine is the physiological stimulus for melatonin production. We are preparing to assess the melatonin rhythm in burn patients. This is all the more important, because burn patients have evidence of altered hypothalamic function, including an altered cortisol rhythm (Vaughan and colleagues, in preparation), and the hypothalamus controls the melatonin rhythm through sympathetic innervation of the pineal gland.

46. Krieger DT, and Rizzo F: Circadian periodicity of plasma 11-hydroxycorticosteroid levels in subjects with partial and absent light perception. *Neuroendocrinol* 8: 165-179, 1971.

47. Dieckhues B: Die Bedeutung der Lichtperzeption durch das Auge auf den Hormonhaushalt des Menschen. *Klin Mbl Augenheilk* 165: 291-296, 1974.

48. Orth DN, Besser GM, King PH, and Nicholson WE: Free-running circadian plasma cortisol rhythm in a blind human subject. *Clin Endocrinol* 10: 603-617, 1979.

49. Akerstedt, T, Froberg, JE, Friberg Y, and Wetterberg L: Melatonin excretion, body temperature and subjective arousal during 64 hours of sleep deprivation. *Psychoneuroendocrinol* 4: 219-225, 1979.

50. Holloway WR, Jr., Tsui HW, Grota LJ, and Brown GM: Melatonin and corticosterone regulation: feeding time or the light: dark cycle? *Life Sciences* 25: 1837-1842, 1979.

PRESENTATIONS/PUBLICATIONS

- 1. Vaughan GM, Harris SC, Allen JP, Delea CS, and Hale SY: Neurologic basis of human melatonin and cortisol rhythms. Military Endocrinologists Meeting, Cincinnati, Ohio, June 17, 1981.**
- 2. Vaughan GM, Harris SC, Allen JP, Delea CS: Comparison of human melatonin and cortisol rhythms. The Endocrine Society Annual Meeting, Cincinnati, Ohio, June 19, 1981.**

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION#	2. DATE OF SUMMARY	REPORT CONTROL NUMBER	
				DA OG 6968	81 10 01	DD-DRLE/ARMY	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY ACTY	6. WORK SECURITY	7. RESEARCH	8. WORK UNIT	9. SPECIFIC DATA- CONTRACTOR ADDRESS	
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10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
	61102A	3M161102B310	BB	303			
11. JUDGMENT							
12. JUDGMENT	STOG 80 - 7.2:5						
11. TITLE (Precede with Security Classification Code)							
(U) Alteration of Host Resistance in Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE REVIEW	
76 10		Cont		DA		C. In-House	
17. CONFIDENTIALITY				18. RESOURCES ESTIMATE			
Not Applicable				PRELIMINARY			
19. DATES/EFFECTIVE:				20. PROFESSIONAL MAN YRS			
EXPIRATION:				1981			
21. NUMBER:				4.0			
22. TYPE:				130			
23. KIND OF AWARD:				1982			
24. CUM. AMT.				5.0			
25. CUM. AMT.				180			
26. RESPONSIBLE S&T ORGANIZATION				27. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Precede with U.S. Address (if different))			
NAME: Basil A. Pruitt, Jr., MD, COL, MC				NAME: Albert T. McManus, Ph.D., MAJ, MSC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221 3411			
28. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMB. "R"			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
29. TECHNICAL OBJECTIVE (Precede with Security Classification Code)							
(U) Tissue Spreading Factors; (U) Rat Model; (U) Infection; (U) Immunostimulants; (U) Virulence Factors; (U) Plasmids; (U) Antibiotic Effects							
30. TECHNICAL OBJECTIVE, 31. APPROACH, 32. PROGRESS (Precede individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To define the microbial basis of opportunistic infection in susceptible burned soldiers. Identify specific mechanisms of decreased host resistance that are targeted by opportunistic pathogens. Develop and evaluate countermeasures.</p> <p>24. (U) The high susceptibility of burned rats to experimental infection with <u>Pseudomonas aeruginosa</u> and <u>Proteus mirabilis</u> will be investigated. The effect of <u>in vitro</u> alterations of specific microbial characteristics on infection will be investigated. Specific antimicrobial and immunostimulator therapies will be examined.</p> <p>25. (U) 8010 - 8109. Summary - A clinical trail of the experimental cephalosporin antibiotic cefsulodin sodium (Abbott) has been initiated. Assessment is in progress. Examination of <u>in vitro</u> sensitivity to cefsulodin following its clinical introduction has shown no evidence of the development of resistance. Eight months post introduction sensitivity is 98.4% for 564 burn patient <u>Pseudomonas aeruginosa</u> isolates. The immunostimulatory agent Glucan has been initially investigated for its value in improving host resistance to pseudomonas infection in burned rats. Intravenous treatment prior to burning and infecting showed no benefit. Local injection into the burn site with delay in bacterial challenge appears to be beneficial.</p>							

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1980 - 30 September 1981

Investigators:

Albert T. McManus, Ph.D., Major, MSC
Jody Corregano, B.A., SP5
Camille L. Denton, M.A.

Reports Control Symbol MEDDH-228(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1980 - 30 September 1981

Investigators: Albert T. McManus, Ph.D., Major, MSC
Jody Corregano, B.A., SP5
Camille L. Denton, M.A.

Reports Control Symbol MEDDH-288(R1)

A clinical trial of the experimental cephalosporin antibiotic cefsulodin sodium (Abbott) has been initiated. Assessment is in progress. Examination of in vitro sensitivity to cefsulodin following its clinical introduction has shown no evidence of the development of resistance. Eight months post introduction sensitivity is 98.4% for 564 burn patient Pseudomonas aeruginosa isolates. The immunostimulatory agent Glucan has been initially investigated for its value in improving host resistance to Pseudomonas infection in burned rats. Intravenous treatment prior to burning and infecting showed no benefit. Local injection into the burn site with delay in bacterial challenge appears to be beneficial.

Tissue Spreading Factors
Rat Model
Infection
Immunostimulants
Virulence Factors
Plasmids
Antibiotic Effects

ALTERATIONS OF HOST RESISTANCE IN BURNED SOLDIERS

EVALUATION OF CEFSULODIN SODIUM

A clinical trial of cefsulodin sodium (Abbott) was initiated during this reporting period. The study is designed to evaluate the safety and efficacy of cefsulodin in the treatment of Pseudomonas aeruginosa infections in burn patients and to compare the safety and efficacy of cefsulodin to agents currently available for the treatment of Pseudomonas aeruginosa infections (Gentamicin, Tobramycin, Amikacin, Carbenicillin or Ticarcillin). Col Basil A. Pruitt, Jr. M.D. is principal investigator. His co-investigators are: A. D. Mason, Jr., M.D., Col William F. McManus, M.D., and Maj Albert T. McManus, Ph.D. This report will summarize the laboratory findings on Pseudomonas aeruginosa isolated during this fiscal year. Clinical evaluation is ongoing and results will be reported in future reports.

A total of 565 strains were examined for antibiotic sensitivity. The sources of patient isolates is presented in Table 1. These data are intended to reflect relative isolation frequency of Pseudomonas aeruginosa in the laboratory. As can be seen, the wound surface (wound swabs and contact plates) was the most common site of isolation (36.57%). The second most common site was the respiratory system (28.27%) which was followed by the flamed biopsy (sub-surface bacteria) (16.25%), which was followed by the urinary tract (8.17%). Pseudomonas aeruginosa was isolated 25 times (4.42%) from blood cultures in a total of 14 patients. All other culture sources resulted in less than 7% of isolates.

Antibiotic sensitivities were determined by disc-diffusion agar overlay technique. Sensitivity was established using the drug manufacturer's recommended zone size criteria. Sensitivity data are presented in Table 2. As can be seen, cefsulodin has maintained a high level of sensitivity (98.4%) despite the clinical exposure of the drug during the isolation period. Other tested cephalosporins were less active. Among the penicillins, Piperacillin was as active as Ticarcillin (95.3% vs 95%). An unexpected finding was a lower frequency of Carbenicillin sensitivity than Ticarcillin sensitivity (40.2% vs 95%). These two antibiotics are commonly thought to have very similar bacterial spectra. Mezlocillin was introduced late in this reporting period. With the examination of only 20 strains, it is too early to judge this drug. High level aminoglycoside resistance was observed (>80%).

The minimum inhibitory concentration of cefsulodin for 460 strains was determined by broth dilution technique. Drug was diluted in Muller-Hinton Broth and inocula were $\approx 10^5$ CFU. The distribution of MIC data are presented in Figure 1. The concentration inhibiting 50% of strains was 1.0 $\mu\text{g/ml}$ which is less than that found during the last reporting period ($\text{MIC}_{50} = 3.1 \mu\text{g/ml}$). The percent of strains sensitive to cefsulodin was 97.17%. This compares well with the disc diffusion data (98.4%).

The distributions of disc diffusion inhibition zones for the examined antibiotics are presented in Figures 2 - 11. Vertical dividing lines in the histograms are the recommended zone diameters representing in vitro sensitivity (S) and resistance (R). The central zone represents intermediate sensitivity (I).

"EXAMINATION OF ANTIBIOTIC SENSITIVITY AS A FUNCTION OF SOURCE OF ISOLATION"

As shown in Table 1, >94% of isolated specimens came from six identifiable sources. The sensitivity of Pseudomonas aeruginosa to selected antibiotics was examined between sources. Data are presented in Table 3. On examination, there appeared to be a relative increase for some antibiotics in sensitivity of strains isolated from the blood and within wounds (flamed biopsy specimens) when compared to all other sources. This possibility was tested by sorting the Pseudomonas sensitivity data on those patients who had positive Pseudomonas blood cultures. The distribution of isolation and patients with positive blood cultures is presented in Table 4. Again it appeared that strains found in the blood and within the wound (flamed biopsy) were more sensitive than Pseudomonas isolates from other sources within the same patient population. This possibility was tested by comparing the combination of blood and biopsy data to all other sources for the same antibiotic. The comparison is presented in Table 5. For unknown reasons, in this group of bacteremic patients, invasive strains are more sensitive to Amikacin, Gentamycin, Carbenicillin and Tetracyclines.

**"EXAMINATION OF PARTICULATE GLUCAN AS A
POSSIBLE NONSPECIFIC IMMUNIZING AGENT IN
EXPERIMENTAL BURN WOUND SEPSIS"**

Glucan is a water insoluble complex polysaccharide material extractable from Saccharomyces cerevisiae. Glucan has been shown to be active as a nonspecific immunostimulant¹.

We have investigated particulate glucan for its possible effects on host resistance in the Walker-Mason Pseudomonas infection model. Initial experiments were to examine the effect of intravenous injection prior to burning and surface inoculation. Male rats (200g) were given 4 mg of Glucan I.V. seven days, 5 days and three days prior to burning and infection. Control groups were a saline group injected in the same course as the Glucan and a burned infected group. Results are presented in Table 6. As can be seen I.V. Glucan at this dose had no positive effect.

Topical application of Glucan was next investigated. The rationale for this approach was that perhaps the failure of systemically administered Glucan was because of the loss of vascular delivery to the wound and that topical Glucan would improve local immune function. Glucan was prepared in a base similar to those used to deliver currently available topical antimicrobial agents, creams containing 5%, 2.5% and 1% w/w were prepared. Creams were applied to rats six hours after burning and infecting. Treatment was continued daily for ten days or until death. Controls included A Glucan free cream group and the controls used in the I.V. experiments. All infected animals died.

The next approach was to introduce Glucan by injection into the burned area and delay Pseudomonas challenge. An experiment was conducted to determine the susceptibility to Pseudomonas infection following infliction of the scald wound. Sixty 200g rats were given 30% full thickness injuries. Groups of ten were inoculated with Pseudomonas aeruginosa strain 1244 (10^8 CFU) at 24 hour intervals for five days. Burned uninfected rats were used as controls. Figure 12 shows that rats lost susceptibility by four days post injury. Glucan was tested by injecting animals immediately following injury with

1. DiLuzio NR, Williams DL, McNamee RB, Edwards BF, and Kitahama A: Comparative Tumor-Inhibitory and Anti-Bacterial Activity of Soluble and Particulate Glucan. Int J. Cancer 1979, Vol 24: 773-779

1ml (10 mg/ml) divided into four injection sites. Controls were injected with 5% dextrose in water. Animals were challenged with 1244 72 hours post burning. Results are presented in Table 7. These preliminary data suggest that Glucan is beneficial. Additionally, they introduce the possibility that local as well as systemic immunity may be a goal in improving host resistance following burn injury.

The following table shows the distribution of *Pseudomonas aeruginosa* isolates by source. The isolates were obtained from various sources, including wound swabs, blood, biopsy, sputum, contact plates, urine, and miscellaneous sources. The table shows the number of isolates and the percentage of the total isolates for each source.

Table 1. Specimen Origins For Antibiotic Surveyed *Pseudomonas aeruginosa* isolated between 1 Oct 1980 and 30 Sep 1981.

<u>SOURCE</u>	<u>NUMBER</u>	<u>PERCENT</u>
Wound Swab	80	14.13
Blood	25	4.42
Biopsy	92	16.25
Sputum	160	28.27
Contact Plates	127	22.44
Urine	46	8.13
Misc. Sources	35	6.36
	565	

Table 2. Antibiotic Sensitivity Patterns of Pseudomonas aeruginosa isolated from burn patients between 1 oct 1980 and 30 Sep 1981.

	<u>PERCENT SENSITIVE</u>	<u>STRAINS TESTED</u>
Aminoglycosides:		
Amikacin	15.9	565
Tobramycin	9.2	402
Gentamycin	17.4	563
Kanamycin	0	564
Neomycin	4.5	554
Streptomycin	0.4	564
Penicillins:		
Ampicillin	0	559
Carbenicillin	40.2	565
Ticarcillin	95	564
Piperacillin	95.3	553
Mezlocillin	60	20
Cephalosporins:		
Moxalactam	86.7	558
Cefotaxime	1.1	179
Cefmenoxime	29.8	302
Cefsulodin	98.4	564
Other Classes:		
Polymyxin B	98.9	564
Colistin	98.8	565
Chloramphenicol	0	565
Tetracycline	3.7	564
Sulfonamides	1.7	543

Table 3. Pseudomonas aeruginosa isolates by Source of Isolation and Percent Sensitivity to Selected Antibiotics.

	<u>Wound Swab</u>	<u>Contact Plate</u>	<u>Blood</u>	<u>Flamed Biopsy</u>	<u>Sputum</u>	<u>Urine</u>	<u>Misc</u>
Aminoglycosides:							
Amikacin	16.3	13.4	32	24.	14.4	10.9	5.6
Gentamycin	21.3	12.6	28	24.	16.3	17.4	5.6
Penicillins:							
Carbenicillin	48.8	24.4	64	41.3	48.1	34.8	27.8
Ticarcillin	98.8	97.7	100	94.6	90.7	87.	100
Cephalosporin:							
Moxalactam	86.3	87.4	88	91.3	81.3	74.	94.4
Tetracyclines	3.8	2.4	16	8.7	0.7	4.4	0

Table 4. Pseudomonas aeruginosa isolates by Source of Isolation and Percent Sensitivity From Patients With Positive Pseudomonas Blood Cultures.¹

	<u>Wound Swab</u>	<u>Contact Plate</u>	<u>Blood</u>	<u>Flamed Biopsy</u>	<u>Sputum</u>	<u>Urine</u>	<u>Misc</u>
Aminoglycosides:							
Amikacin	16	14.3	32	31.9	17.8	5.9	5.9
Gentamycin	24	21.4	28	41.	25.9	23.6	5.9
Penicillins:							
Carbenicillin	40	25	64	56.9	48.4	47.1	23.6
Ticarcillin	100	100	100	97.8	88.8	100	100
Cephalosporin:							
Moxalactam	64	75	88	93.2	77.4	82.4	100
Tetracyclines	4	7.1	16	16.	1.7	11.8	0

¹ 25 Positive Blood Cultures in 14 Patients, 1 Oct 1980 - 30 Sep 1981.

Table 5. Relative Antibiotic Sensitivity of Pseudomonas aeruginosa Isolated From Blood and Biopsies When Compared to all Other Sources of Isolation in Patients with Pseudomonas Bacteremia.¹

HYPOTHESIS: Blood and biopsy isolates were more sensitive to selected antibiotic than strains isolated from all other sources.

<u>ANTIBIOTIC</u>	<u>TRUE</u>	<u>FALSE</u>	<u>SIGNIFICANCE</u> ²
Amikacin	X		P = .002
Gentamycin	X		P = .029
Carbenicillin	X		P = .006
Moxalactam		X	P = .39
Tetracyclines	X		P = .006

1. 25 Episodes in 14 Patients 1 Oct 1980 - 30 Sep 1981.
2. Chi Square Analysis.

Table 6. Effect of Intravenous Particulate Glucan* On Experimental Pseudomonas Burn Wound Sepsis**

TREATMENT	MORTALITY											TOTAL DEAD
	Days Postburn Inoculation											
	0	1	2	3	4	5	6	7	8	9	10	
Glucan + infection	-	-	-	-	-	1	3	11	1	-	-	16/16
Saline + infection	-	-	-	1	1	-	2	-	4	3	6	17/17
Infection alone	-	-	-	-	-	1	1	4	5	2	5	18/18

*Glucan (4 mg) I.V. 7, 5 and 3 days prior to burning and inoculation

**200 gram rats 30% scald inoculated with 10^8 CFU strain 1244

Table 7. Effect of Intraeschar Injection of Glucan¹ On Susceptibility to Fatal Pseudomonas Burn Wound Sepsis

<u>TREATMENT</u>	<u>SURVIVAL</u>		<u>TOTAL</u>
	<u>EXP 1</u>	<u>EXP 2</u>	
Glucan (10 mg)	5/6	6/10	11/16
Dextrose (5%)	0/6	2/10	2/16

¹Glucan was injected below the burn site following 30% scalding of 200g rats.

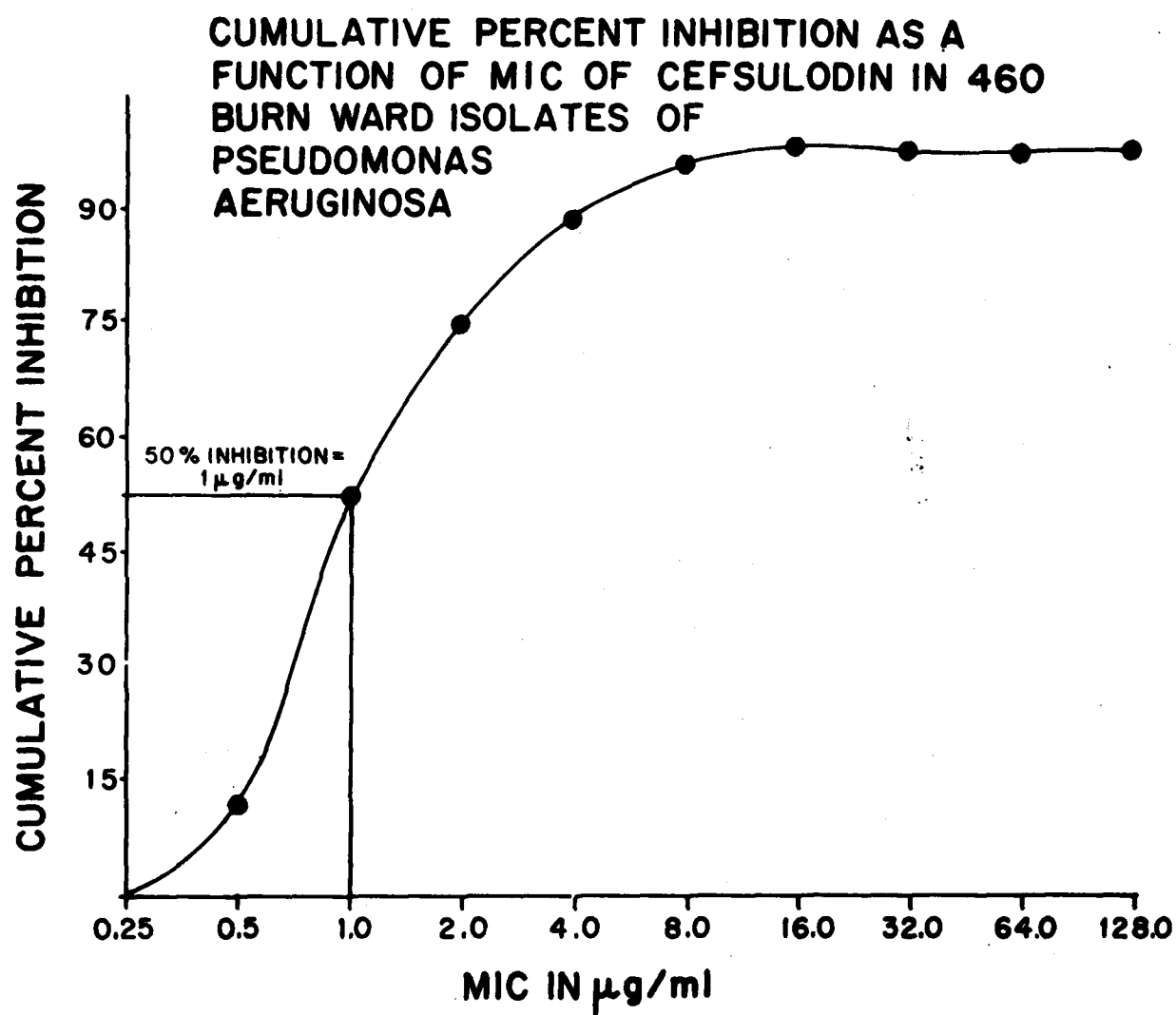
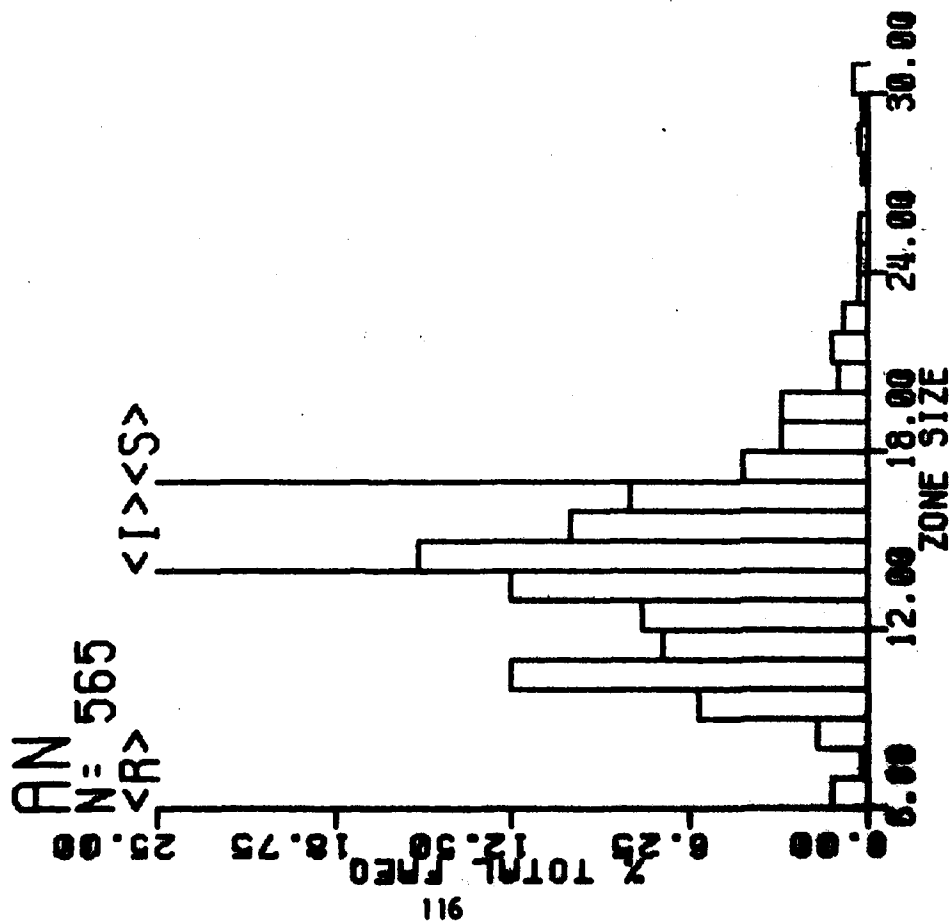
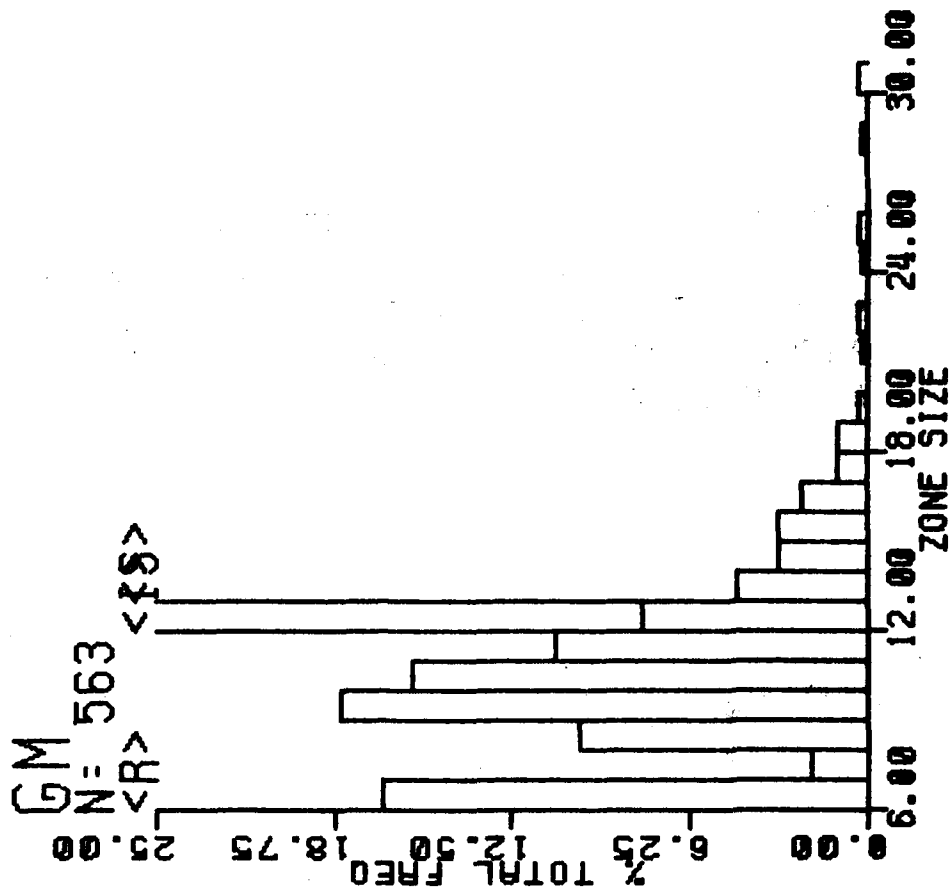


FIGURE 1

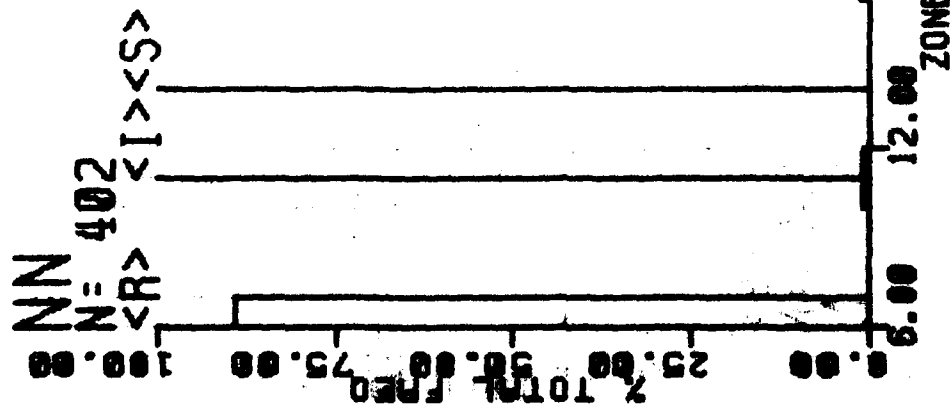


A. Frequency Distribution of zones of inhibition using Amikacin sensitivity disc AN-30 (30 µg)

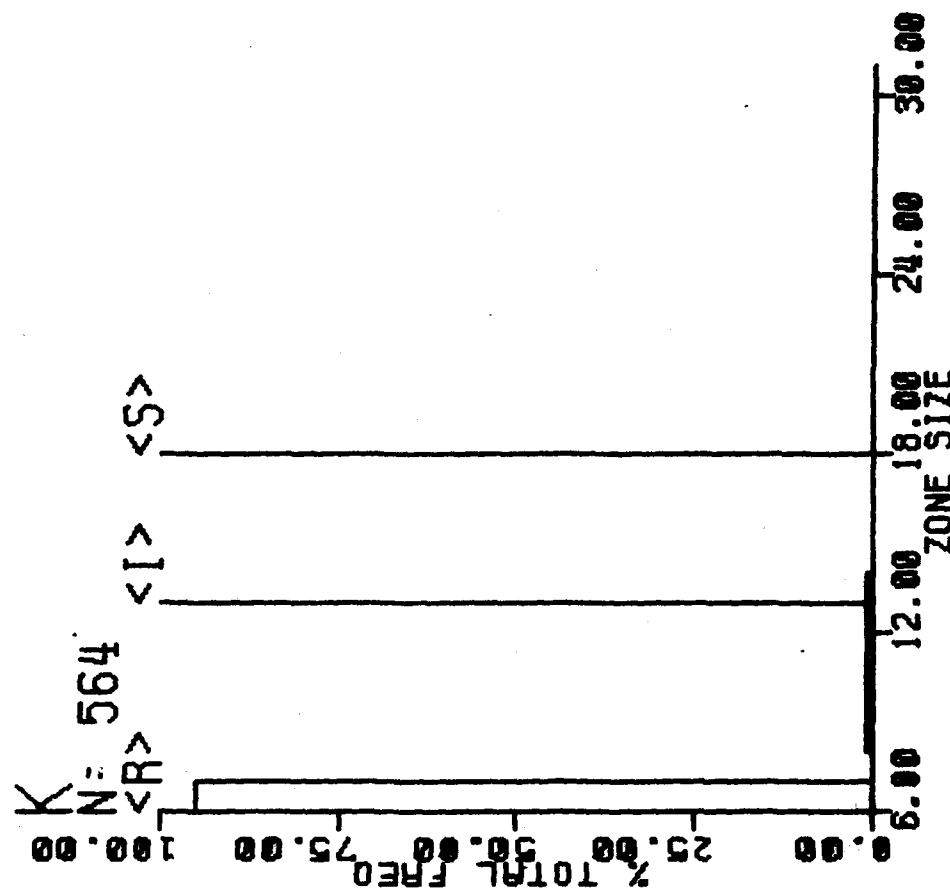


B. Frequency distribution of zones of inhibition using Gentamicin sensitivity disc GM-10 (10 µg)

FIGURE 2



A. Frequency distribution of zones of inhibition using Tobramycin sensitivity disc NN-10 (10 µg)



B. Frequency distribution of zones of inhibition using Kanamycin sensitivity disc K-30 (30 µg)

FIGURE 3

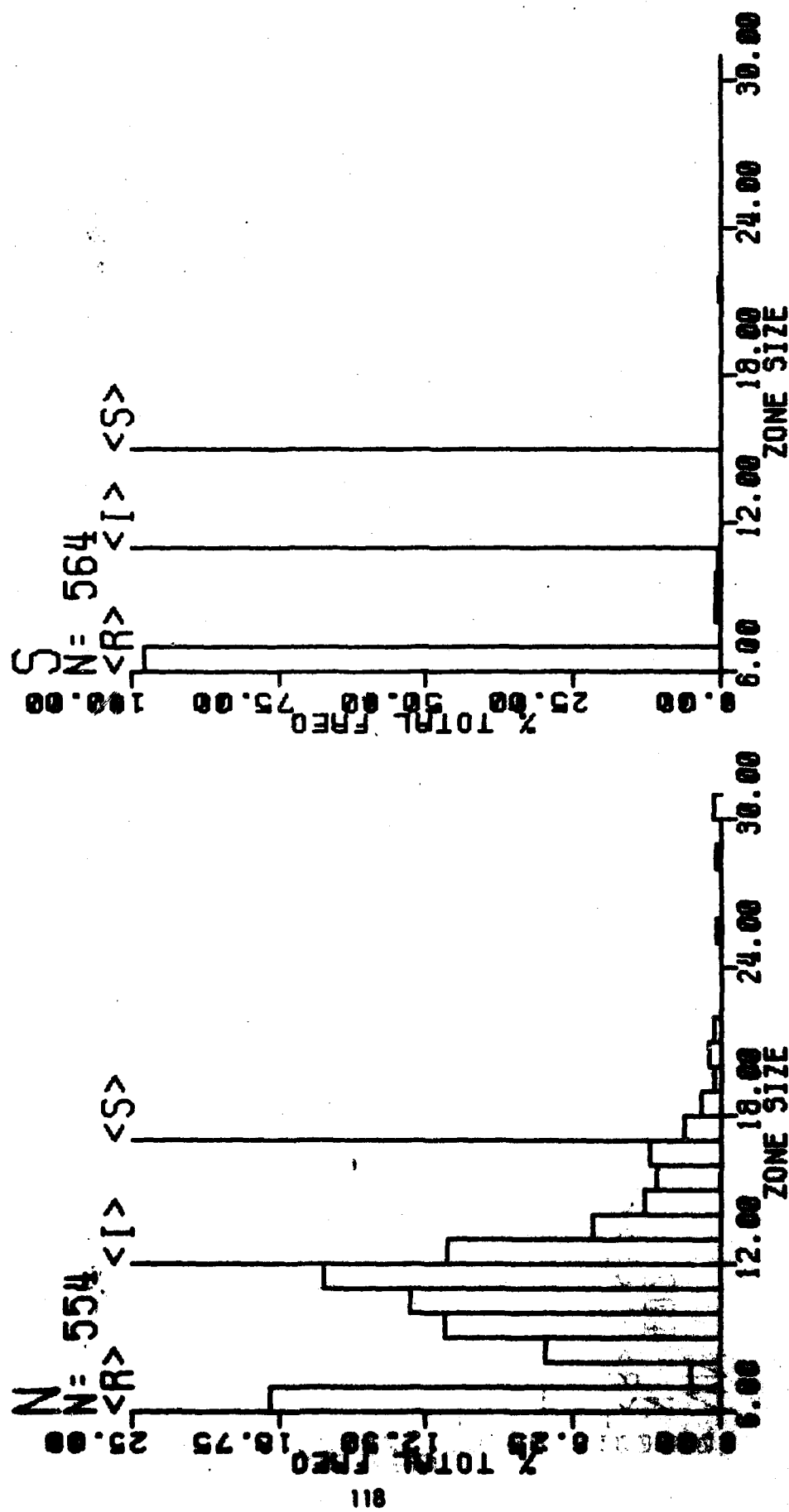
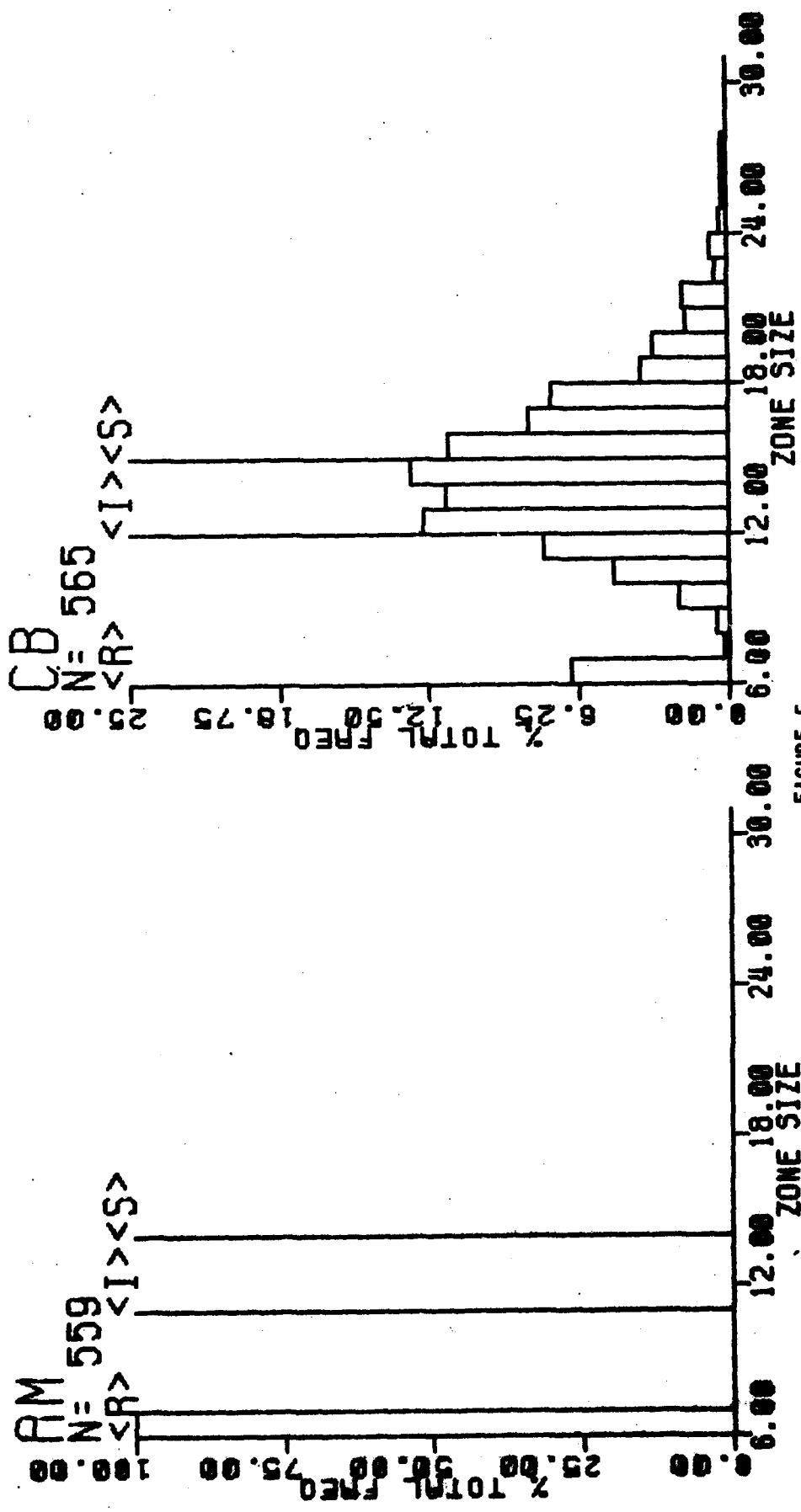


FIGURE 4

A. Frequency distribution of zones of inhibition using Neomycin sensitivity disc N-30 (30 µg)

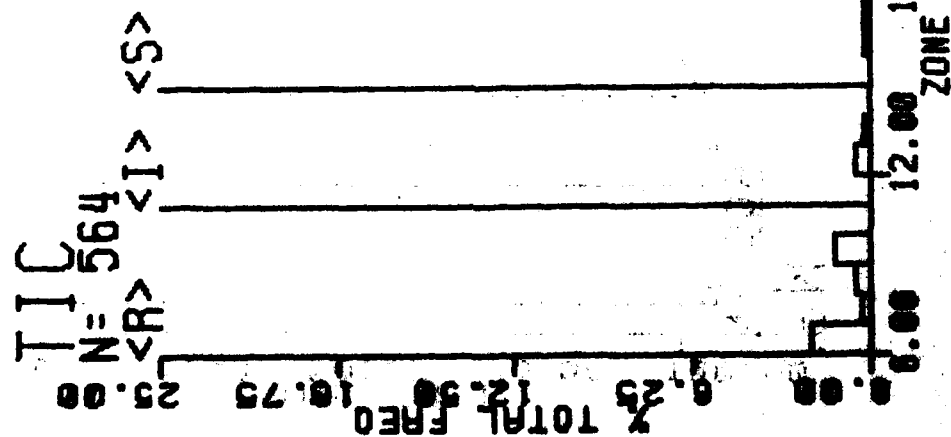
B. Frequency distribution of zones of inhibition using Streptomycin sensitivity disc S-10 (10 µg)



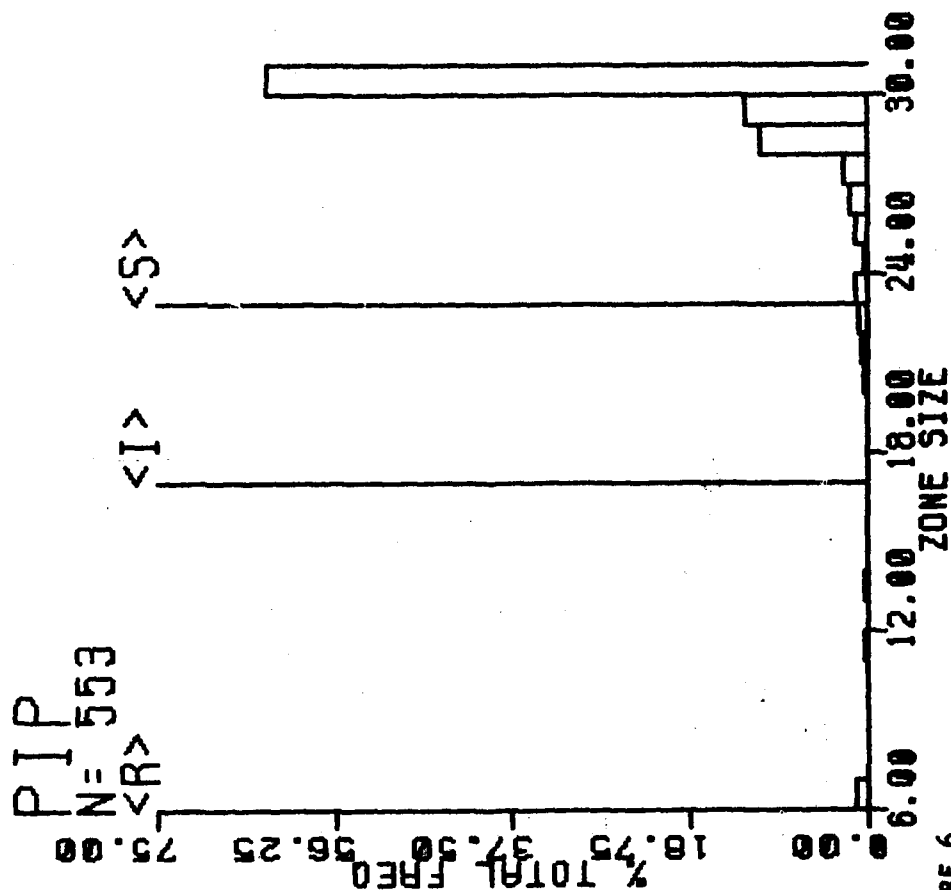
A. Frequency distribution of zones of inhibition using Ampicillin sensitivity disc AM-10 (10 µg)

B. Frequency distribution of zones of inhibition using Carbenicillin sensitivity disc CB-100 (100 µg)

FIGURE 5



A. Frequency distribution of zones of inhibition using Ticarcillin sensitivity disc TIC-75 (75 µg)



B. Frequency distribution of zones of inhibition using Piperacillin sensitivity disc PIP-100 (100 µg)

FIGURE 6

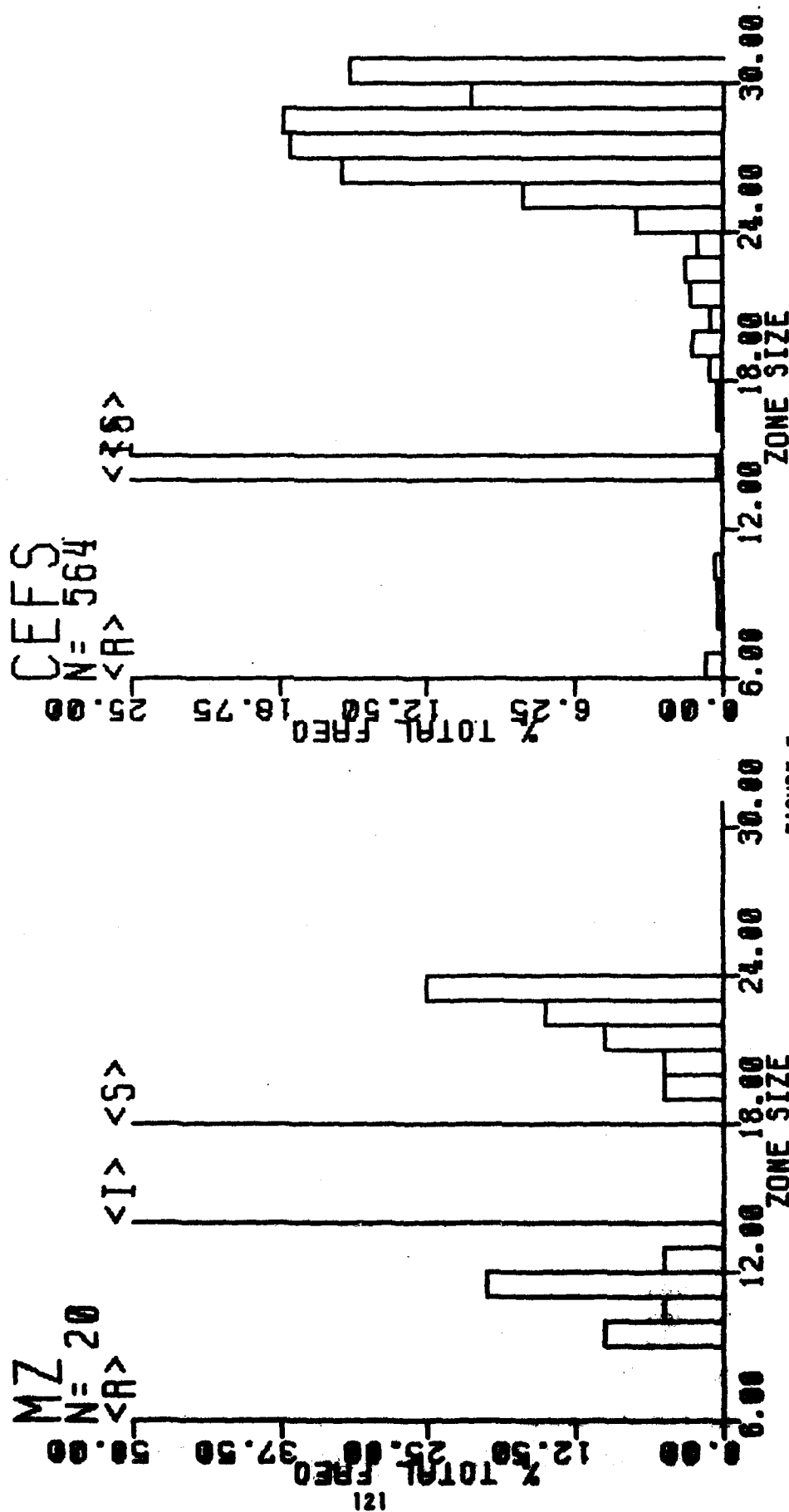
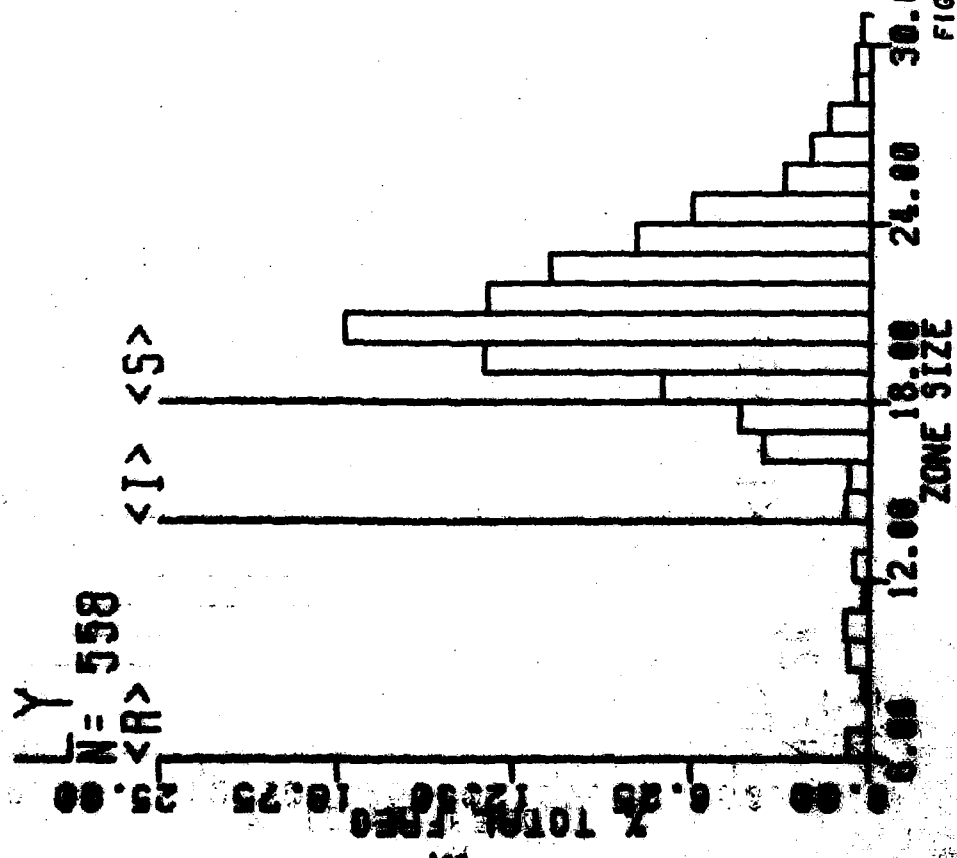


FIGURE 7

A. Frequency distribution of zones of inhibition using Mezlocillin sensitivity disc MZ-75 (75 µg)

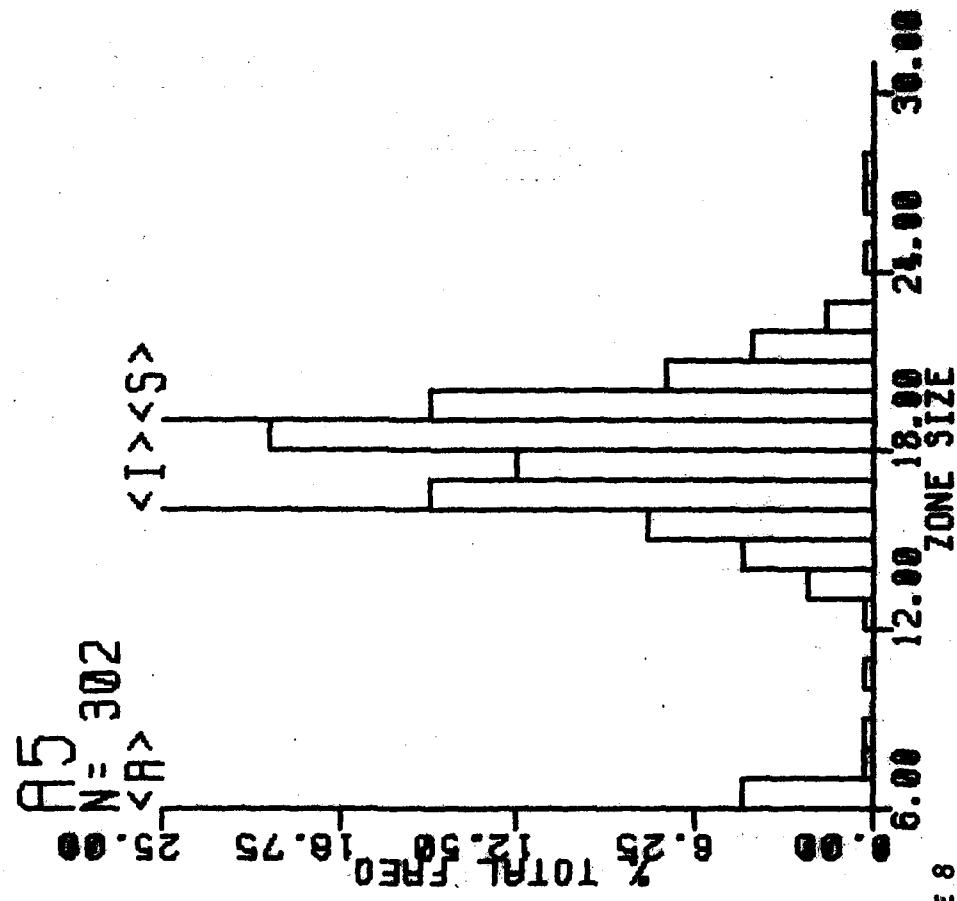
B. Frequency distribution of zones of inhibition using Cefsulodin sensitivity disc CEF-30 (30 µg)

LY
N= 558
<I> <R>



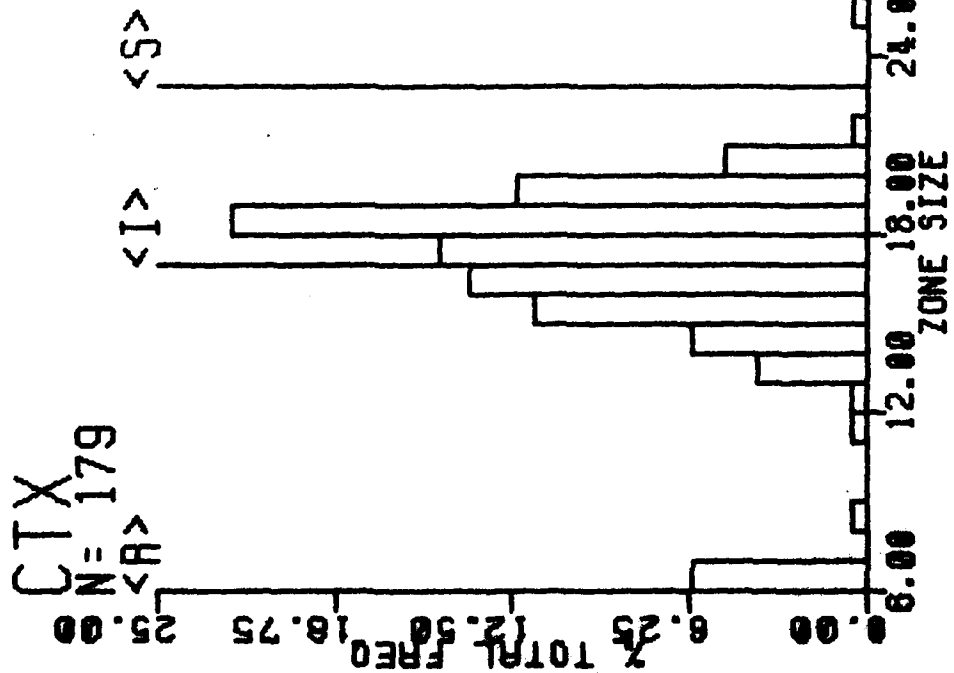
A. Frequency distribution of zones of inhibition using Moxalactam disodium sensitivity disc MOX-30 (30 µg)

A5
N= 302
<I> <R>

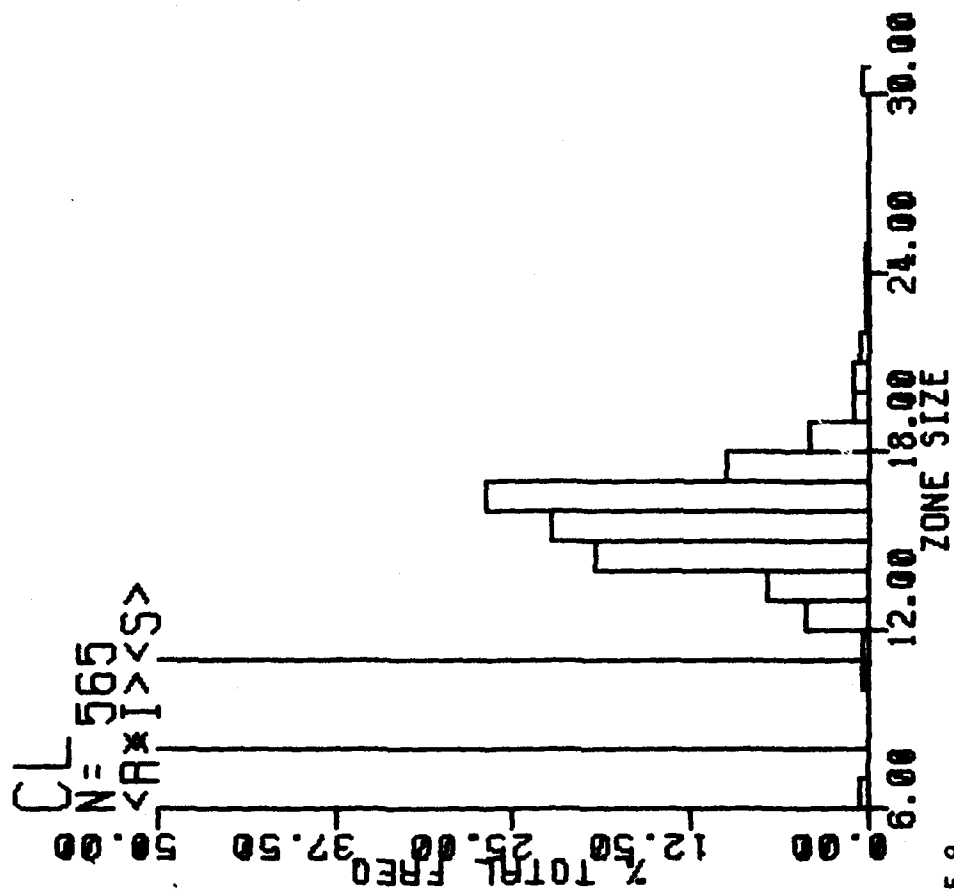


B. Frequency distribution of zones of inhibition using Cefmenoxime sensitivity discs A5-30 (30 µg)

FIGURE 8

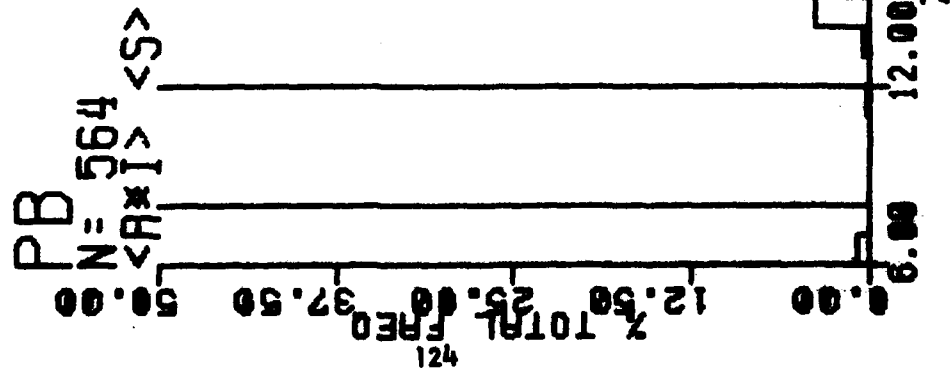


A. Frequency distribution of zones of inhibition using Cefotaxime sensitivity disc CTX-30 (30 µg)

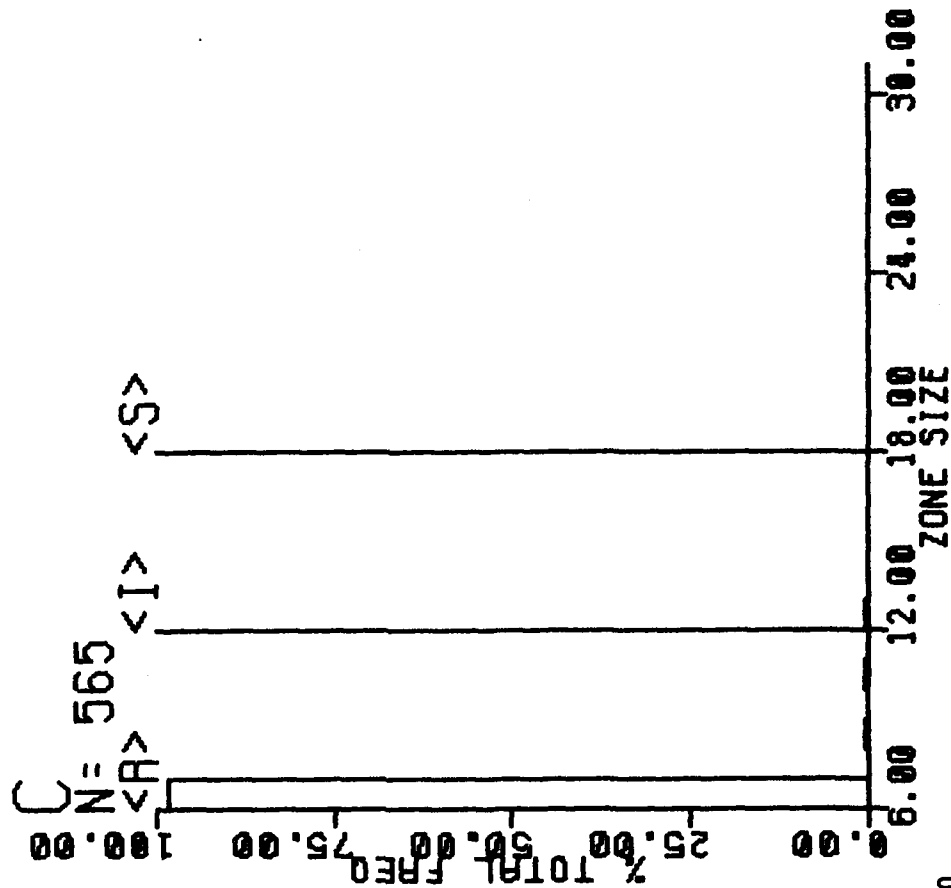


B. Frequency distribution of zones of inhibition using Colistin sensitivity disc CL-10 (10 µg)

FIGURE 9



A. Frequency distribution of zones of inhibition using Polymyxin-B sensitivity disc PB-300 (300 u)



B. Frequency distribution of zones of inhibition using Chloramphenicol sensitivity disc C-30 (30 ug)

FIGURE 10

**EFFECT OF TIME OF SURFACE INOCULATION^{*} ON SUSCEPTIBILITY
TO FATAL PSEUDOMONAS BURN WOUND SEPSIS OF THE 30 %
SCALDED RAT**

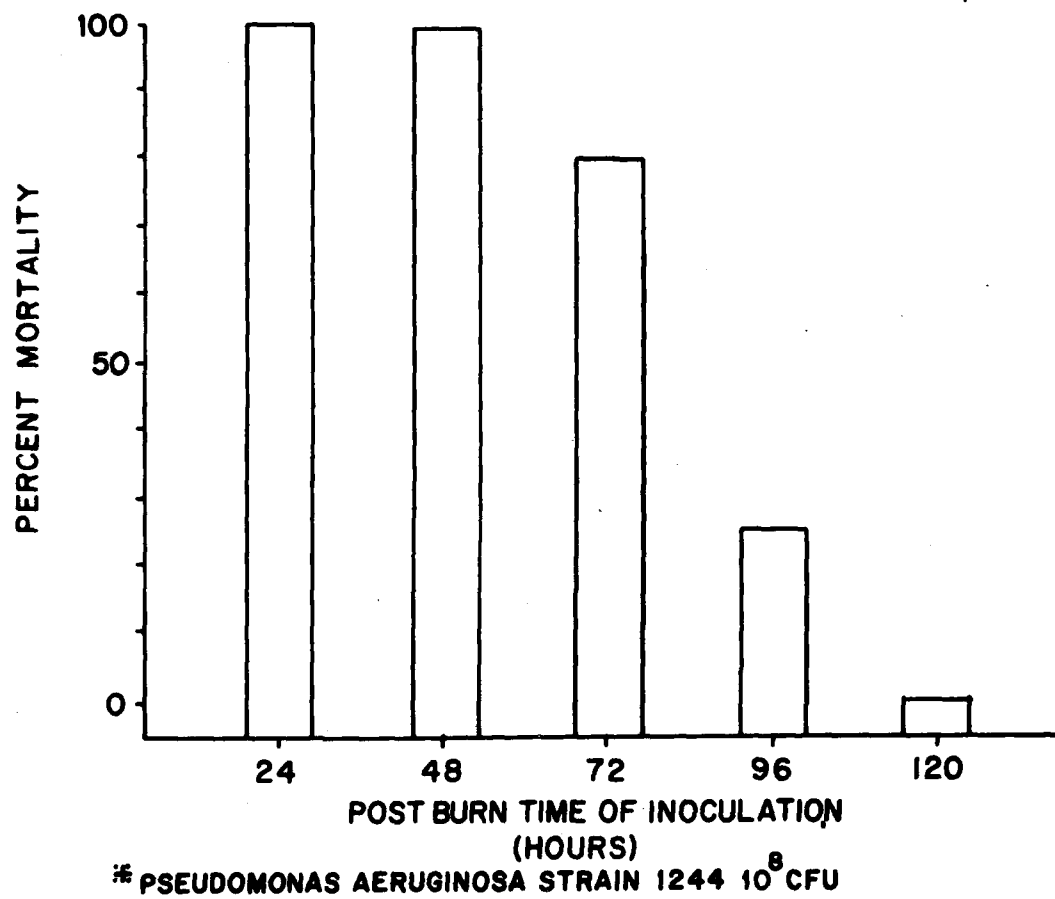


FIGURE 12

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)436	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISC'D INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS ^a	9. LEVEL OF SUM ^a
80 10 01	K. COMP	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3M161102BS10		BB		305	
b. XXXXXXXXXX							
c. XXXXXXXXXX	STOG 80-7.2:5						
11. TITLE (Precede with Security Classification Code) ^a (U) Evaluation of Synthetic Sheeting as Operating Room Drapes Material For Use in a Military Burn Unit (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
70 07		81 12		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. FUND (in thousands)	
a. DATES/EFFECTIVE:		EXPIRATION:		PRECEDENCE			
b. NUMBER ^a				FISCAL YEAR		11	
c. TYPE:		d. AMOUNT:		CURRENT		12	
e. KIND OF AWARD:		f. CUM. AMT.		1982		0.2	
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME ^a US Army Institute of Surgical Research				NAME ^a US Army Institute of Surgical Research			
ADDRESS ^a Ft Sam Houston, Texas 78234				ADDRESS ^a Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Precede with U.S. Academic Position)			
NAME: Basil A. Pruitt, Jr., MD, COL, MC				NAME ^a Basil A. Pruitt, Jr., MD, COL, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2720			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Robert B. Lindberg, Ph.D.			
				NAME:			
				POC: DA			
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Precede individual paragraphs identified by number. Precede text of each with Security Classification Code.)							

23. (U) Evaluation in terms of draping characteristics, absorbency, physician acceptance, and bacterial barrier qualities of a Spunbonded Olefin-cellulosic Laminated sheeting as surgical drapes and gowns. A decrease in bacterial seeding of operative wounds via drapes will minimize postoperative wound infections decreasing subsequent morbidity and mortality in injured troops.

24. (U) Laboratory assessment of bacterial barrier properties of synthetic sheeting. Clinical use of drapes on burn patients to determine surgeon acceptability. Photographic documentation of draping characteristics, absorbency, and "run-off". Pre-and post-operative cultures at margin of operative field. Temperature monitoring to determine heat transmission characteristics.

25. (U) 8010 - 8109. The bacterial barrier function of 10 samples of non-woven synthetic drape materials has been further tested using the assay developed in this laboratory. Differences between organism penetrating ability and drape penetrability were both evident. The Klebsiella pneumoniae and Pseudomonas aeruginosa test strains penetrated more consistently than the other four test organisms. The least resistant drape material permitted penetration of bacteria at 94.5% of test sites, while bacterial penetration occurred at only 7.1% of test sites

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACRONYM	2. DATE OF SUMMARY	REPORT NUMBER (DD-Form 1498)	
				DA OD 6978	81 10 01		
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. RESEARCH	8. BUDGET ESTIMATE	9. SPECIAL DATA - CONTRACTOR AGENCY	
80 10 01	K. COMP	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO A. WORK UNIT	
10. NO./CODES		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
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b. XXXXXXXXXX							
c. XXXXXXXXXX		STOG 80-712:5					
11. TITLE (Precede with Security Classification Code) (U) Evaluation of Synthetic Sheeting as Operating Room Drape Material For Use in a Military Burn Unit (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
70 07		81 12		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PREVIOUS		b. FUNDS (In thousands)	
c. NUMBER:				FISCAL YEAR		11	
d. TYPE:				1981		0.2	
e. KIND OF AWARD:				1982		12	
f. CUM. AMT.							
20. RESPONSIBLE S&T ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr., MD, COL, MC				NAME: Basil A. Pruitt, Jr., MD, COL, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2720			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Robert B. Lindberg, Ph.D.			
				POC: DA			
23. SUMMARY (Precede with Security Classification Code)							
(U) Military Burn Unit; (U) Operating Room Based Infections; (U) Surgical Drapes; (U) Surgical Gowns							
24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>in the best drape material. Drape characteristics were the predominant determinant of bacterial penetration. Since all of the synthetic sheeting materials functioned as ineffective microbial barriers in an absolute sense, none can be recommended for clinical use at this time and the project has been terminated.</p>							

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, MILITARY BURN RESEARCH

**REPORT TITLE: EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM
DRAPE MATERIAL FOR USE IN A MILITARY BURN UNIT**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**Basil A. Pruitt, Jr., MD, FACS, Colonel, MC
Robert B. Lindberg, PhD
Arthur D. Mason, Jr., MD**

Reports Control Symbol MEDDH-288(RI)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3M161102BS10-00, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM
DRAPE MATERIAL FOR USE IN A MILITARY BURN UNIT

US Army Institute of Surgical Research, Brooke Army Medical
Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1980 - 30 September 1981

Reports Control Symbol MEDDH-288(RI)

The bacterial barrier properties of 10 samples of non-woven synthetic surgical drape material were tested using an assay developed at this Institute. The transmission of each of five different bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Serratia marcescens*) suspended in liquid culture media and inoculated on samples of each of the 10 drape materials was determined in seven replicate trials of each material.

Differences between organism penetrating ability and drape penetrability were both identified. The *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* test strains penetrated the drape materials with greater frequency than the other test organisms. The synthetic drape materials, on the basis of bacterial penetration, could be classified into those showing little bacterial barrier function and those materials showing variable resistance to bacterial penetration. The penetration of bacteria at inoculation sites on those materials having little barrier property, ranged from 78% to 95%. In those materials showing some bacterial barrier property, penetration occurred at from 68% of test sites to as few as 7% of test sites in that material showing the greatest resistance to microbial penetration. The physical characteristics of the drape material appeared to be the predominant determinant of microbial penetration. None of these nonwoven synthetic surgical drape materials functioned as an absolute microbial barrier. The composition and the processing which appear to compromise the microbial barrier properties of these materials are apparently necessary in order to produce a material with clinically acceptable draping characteristics. None of these materials can be recommended for clinical use at this time and the project is terminated.

EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM DRAPE MATERIAL FOR USE IN A MILITARY BURN UNIT

Standard surgical drapes made of muslin or cotton, once they become moistened in the course of a surgical procedure permit ready penetration of bacteria and other microorganisms. Single use disposable synthetic drape materials which are now used in over half of all operative procedures performed in the United States are considered to possess bacterial barrier properties superior to those of cloth drape materials and prevent microbial migration into the surgical field and the operative wound. The synthetic drape materials which are easily disposable show decreased linting and, in earlier studies, did show good resistance to bacterial penetration. The poor draping characteristics of the earlier synthetic drape materials and the fact that such materials permitted quantitative run-off of liquids from the operative field onto the surgeon limited their clinical acceptance. Consequently, the composition and the processing used in the production of the nonwoven synthetic drapes has been modified to improve the draping characteristics and "soften" the material. Such alterations of the nonwoven fabrics does indeed improve their draping characteristics but has adversely affected their bacterial barrier function. Testing of newer forms of "softened" nonwoven synthetic drape materials has been carried out to evaluate the adequacy of their microbial barrier function.

Methods

Disks of the synthetic drape materials, 90 mm in diameter, were cut, gas sterilized, and placed on the surface of blood agar culture plates. The top-bottom orientation designated for each sample of drape material was observed with the upper surface of the disk corresponding to the side of the drape which would not be in contact with the surface of the patient. Six drops of an overnight TSS broth culture of each bacterial strain used for testing were placed at equidistant intervals on each disk. The spacing permitted differentiation of individual areas of growth of the test organism, if it penetrated the test material. The drops of bacteria-containing broth were left in position on the drape material for four hours at room temperature, following which any remaining culture liquid was removed using a micropipette. The disk of draping material was then carefully removed and the plate incubated overnight at 37° C. Penetration of the drape material was considered to have occurred if growth of the bacterial test strain was apparent at any site where it had rested on the material. If confluent growth occurred which encompassed two inoculation sites, each of the sites involved was considered as a site of penetration although it would be theoretically possible for organisms from a single site of penetration to spread over adjacent sites under the disk of drape material. Each of 10 drape material samples was tested for penetration by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Serratia marcescens*. Seven replicate

trials of each drape material for each organism were carried out - a total of 2,100 inoculation sites.

Results

The percentage of penetration of each drape material by all test strains is shown in the Table. There is identifiable microbial genus-specific variation in penetrability within drape materials, but a greater difference was observed between materials in terms of overall microbial penetration. The percentage of inoculation sites at which bacterial penetration occurred was least in sample number 9 with transmission occurring at 7.1% of inoculation sites. The per cent of inoculation site penetration in the other samples ranged from 17.1% to 94.5% with the highest rate of penetration occurring in sample number 7.

Discussion

The 10 samples of nonwoven synthetic drape material which were tested showed considerable variation in microbial penetration which ranged from 7.1% for sample number 9 to 94.5% for sample number 7. Analysis of the results indicates that there are differences both between drape material samples and between organism penetrability with the former being of statistical significance. Drape material penetration was greatest by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* organisms and least by *Staphylococcus aureus*. On the basis of percentage of inoculation sites penetrated, two groups of drape materials can be identified, i.e., those with little or no resistance to bacterial penetration and those with variable resistance to bacterial penetration. The rate of penetration in the materials showing little resistance to microbial transmission ranged from 78% for sample number 8 to 94% for sample number 7. The rate of penetration of inoculated bacteria in those drape materials showing some barrier function ranged from 7.1% to 67.6%. In addition to sample number 9, which permitted penetration at only 7.1% of inoculation sites, sample number 1 permitted penetration at only 18.1% of inoculation sites and sample number 6 permitted penetration at only 19.5% of inoculation sites. The mean incidence of penetration in the three materials showing the least resistance to bacterial penetration was 87.9% while the mean penetration in the samples showing the greatest resistance to bacterial penetration was only 14.9%.

These further studies confirm earlier tests indicating that drape composition is of importance for the bacterial barrier function of nonwoven synthetic operating room drapes. None of the materials tested in this group of samples performed as well as certain previously tested samples and little progress in producing a drape possessing an absolute bacterial barrier properties has been made. Alterations in drape composition and processing which appear to be necessary to produce material with

clinically acceptable fabric characteristics appear to diminish the resistance of the materials to bacterial penetration. Since none of the materials tested functioned as an absolute microbial barrier, none can be recommended for clinical use at this time and the project is terminated.

TABLE 1

SUMMARY TABLE

PROPORTION OF TEST STRAINS PENETRATING DRAPE MATERIALS

Sample No.	ORGANISM AND NUMBER OF TEST SITES PENETRATED (%)					Total	Total % Penetration
	Pseudo. aerug	Staph. aureus	E. Coli	Klebsiella Pneumoniae	Serratia Marces.		
1	8/42(19.0)	4/42(9.5)	10/42(23.8)	11/42(26.2)	5/42(11.9)	38/210	18.1
2	5/42(11.9)	8/42(19.0)	9/42(21.4)	18/42(42.9)	6/42(14.3)	46/210	21.9
3	40/42(95.2)	36/42(85.7)	41/42(97.6)	40/42(95.2)	35/42(83.3)	192/210	91.4
4	31/42(73.8)	12/42(28.6)	25/42(59.5)	8/42(19.0)	16/42(38.1)	92/210	43.8
5	21/42(50.0)	2/42(4.8)	8/42(19.0)	25/42(59.5)	10/42(23.8)	66/210	31.4
6	4/42(9.5)	3/42(7.1)	12/42(28.6)	17/42(40.5)	5/42(11.9)	41/210	19.5
7	42/42(100.0)	40/42(95.2)	41/42(97.6)	35/42(83.3)	40/42(95.2)	198/210	94.3
8	42/42(100.0)	28/42(66.7)	30/42(71.4)	35/42(83.3)	29/42(69.0)	164/210	78.1
9	4/42(9.5)	3/42(7.1)	1/42(2.4)	5/42(11.9)	2/42(4.8)	15/210	7.1
10	26/42(61.9)	21/42(50.0)	25/42(59.5)	39/42(92.9)	31/42(73.8)	142/210	67.6
Total	223/420	157/420	202/420	233/420	179/420	994/2100	
Total % Penetration	53.1	37.4	48.1	55.5	42.6		47.3

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL	
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3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORIGIN INSTN	8B. SPECIFIC DATA - CONTRACTOR ACCESS	8. LEVEL OF SW
80 10 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES:		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
A. PRIMARY		61102A		3H161102B510		BB	
B. XXXXXXXXX						301	
C. XXXXXXXXX		STOG 80 - 7.2:5					
11. TITLE (Precede with Security Classification Code)							
(U) Studies of Infection and Microbiologic Surveillance of Troops With Thermal Injury (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
76 10		Cont		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE			
Not Applicable				A. PROFESSIONAL MAN YRS			
A. DATES/EFFECTIVE:				B. FUNDS (in thousands)			
EXPIRATION:				PRECEDENCE			
B. NUMBER:				FISCAL YEAR			
C. TYPE:				1981			
D. AMOUNT:				3.0			
E. CUM. AMT.				178			
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				Microbiology Branch			
RESPONSIBLE INDIVIDUAL				ADDRESS: Ft Sam Houston, Texas 78234			
NAME: Basil A. Pruitt, Jr., MD, COL, MC				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
TELEPHONE: 512-221-2720				NAME: Robert B. Lindberg, Ph.D.			
				TELEPHONE: 512-221-2018			
				SOCIAL SECURITY ACCOUNT NUMBER:			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
FOREIGN INTELLIGENCE NOT CONSIDERED				NAME: Albert T. McManus, Maj, MSC, Ph.D.			
				NAME: 512-221-3411			
				POC: DA			
22. RESEARCH PURPOSES (State and Security Classification Code) (U) Pseudomonas; (U) Klebsiella; (U) Staphylococcus; (U) Young Infection; (U) Antibiotic Resistance; (U) Sepsis; (U) Topical Chemotherapy; (U) Humans							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Burns constitute a large component of military injuries sustained in combat. Military relevance of this research lies in the fact that infection and ensuing sepsis are major problems among burned soldiers. Control of surface infection is a major objective, and species of organisms causing sepsis, epidemiology, response of significant species to topical chemotherapy modalities, and relation of antibiotics to sepsis control are major study areas.</p> <p>24. (U) Culture of human wounds, tissues and body fluids are carried out with precise strain speciation and differentiation being employed. Virulence is assessed in burn wound models which are used to assess experimental drugs, both topical and systemic.</p> <p>25. (U) 8010 - 8109. <u>Pseudomonas aeruginosa</u> continued as the principal bacterial species isolated (749 strains). Other significant species were: <u>Providencia stuartii</u> (475); <u>Staphylococcus aureus</u> (332), <u>Streptococcus viridans</u> (266); <u>Klebsiella pneumoniae</u> (206); <u>Escherichia coli</u> (204) and <u>Staphylococcus epidermidis</u> (168). Blood culture results were positive for 139 specimens of 1499 submissions. Of positive blood cultures, 43 (30.9%) were <u>Ps. aeruginosa</u>, 31 (22.3%) were <u>Prov. stuartii</u>, 18 (12.9%) were <u>Staph epidermidis</u>; 11 (7.9%) were <u>Staph. aureus</u> and 10 (7.1%) were <u>Kleb. pneumoniae</u>. A total of 20 species were isolated from blood cultures. Predominant wound flora isolates were in decreasing</p>							

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL	
				DA OG 6972	81 10 01	DD-DRAE(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORIGIN INSTN	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES:		PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY		61102A	3M161102BS10	BB	301		
b. XXXXXXXX							
c. XXXXXXXX		STOG 80 -	7.2:5				
11. TITLE (Precede with Security Classification Code)							
(U) Studies of Infection and Microbiologic Surveillance of Troops With Thermal Injury (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
76 10		Cont		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE			
Not Applicable				PRECEDENCE			
a. DATES/EFFECTIVE:		EXPIRATION:		FISCAL YEAR	a. PROFESSIONAL MAN YRS		b. FUNDS (in thousands)
b. NUMBER:		c. TYPE:		1981	3.0		178
d. KIND OF AWARD:		f. CUM. AMT.		1982	2.5		188
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Fort Sam Houston, Texas 78234				ADDRESS: Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish DDAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr., MD, COL, MC				NAME: Robert B. Lindberg, Ph.D.			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2018			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Albert T. McManus, Maj, MSC, Ph.D.			
				NAME: 512-221-3411 POC: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Wound Infection; (U) Antibiotic Resistance; (U) Pseudomonas; (U) Klebsiella; (U) Staphylococcus; (U) Humans; (U) Sepsis; (U) Topical Chemotherapy;							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>frequency: <u>Ps. aeruginosa</u> 309 (30.9%); <u>Prov stuartii</u> 219 (21.9%); <u>Staph aureus</u> 67 (6.7%) and <u>Staph epidermidis</u> 66 (6.6%). Predominant respiratory tract flora isolates included: <u>Ps. aeruginosa</u> 249 (17.8%); <u>Staph aureus</u> 224 (16%); <u>Strep viridans</u> 224 (16%); <u>Prov stuartii</u> 133 (9.5%); and <u>Kleb pneumoniae</u> 110 (7.8%). <u>In vitro</u> sensitivity of <u>Ps. aeruginosa</u> to mafenide acetate (sulfamylon R) has remained uniformly high. Examination of virulence using the burned rat model has shown a low frequency of wound invasiveness using isolates of <u>Ps. aeruginosa</u> from the patient population.</p>							

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

**REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF
TROOPS WITH THERMAL INJURY -- ANTIBIOTIC SENSITIVITY
OF CURRENT MILITARY BURN PATIENT FLORA**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**Robert B. Lindberg, Ph.D.
Jack R. Henderson, Ph.D.
Susan J. Constable, SSG
Gloria Bailey, SP5**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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vo hundred and eight strains of bacteria, including 14 species, were
t by MIC procedure for sensitivity in 1980-1981. Sources were pri-
m blood culture and wound biopsy; sputum cultures made up the re-
m . Pseudomonas aeruginosa and Providencia stuartii were the
p al species represented from blood cultures. For unknown reasons
a lpitous drop in the number of strains of Staphylococcus aureus from
b culture occurred. The few strains of Staphylococci tested were
m nsitive to the whole antibiotic spectrum than were any previous
s ested. Pseudomonas aeruginosa strains became somewhat more sensi-
t gentamicin, tobramycin and amikacin during the year, as they did
t cacyclines. Klebsiella pneumoniae and Enterobacter cloacae were
m ely sensitive to aminoglycosides and to tetracyclines. Provi-
d stuartii was sensitive in 58% of isolates to amikacin and to no
o antibiotics.

A otic sensitivity
P onas
B ounds
P encia stuartii

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS
WITH THERMAL INJURY -- ANTIBIOTIC SENSITIVITY OF CURRENT
MILITARY BURN PATIENT FLORA

Antibiotics are an indispensable part of the armamentarium for control of infection in burn patients. However, the opportunistic nature of much of the burn flora has as a corollary a heterogeneous spectrum of resistance patterns. This is, perforce, the flora involved in invasion or potential invasion of viable tissue adjacent to the burn wound. It has been shown to be counterproductive to assay sensitivity of the entire colonizing flora. Only those strains which exhibited invasive potential on wound biopsy or those recovered from blood culture were routinely tested for MIC against a standard battery of selected antibiotics.

It has been pointed out in another section of this report that the number of patients exhibiting bacterial invasion appeared to diminish again in 1980-1981, a trend first commented on in 1979-1980.

STRAINS TESTED AND ANTIBIOTICS EMPLOYED

Fourteen species of bacteria and 208 strains were tested by tube dilution Minimum Inhibitory Concentration (MIC) technic. The number of strains tested was the smallest observed in the past 15 years. This decrease is not solely explained on the basis of a smaller number of patients admitted; there was a continuing drop in the percentage of patients who exhibited sepsis. A comparison of numbers of strains of the major species involved is shown in Table 1. In this year, only two species were tested in significant numbers: Pseudomonas aeruginosa and Providencia stuartii. The predominance of Pseudomonas is of long standing, but Providencia had not been seen in large numbers since 1974. Enteric species which have been important in nosocomial outbreaks, especially Klebsiella pneumoniae and Enterobacter cloacae, were recovered in only a few patients in 1980-1981.

TESTING SYSTEM FOR ANTIBIOTICS

The test battery of antibiotics routinely used in this Institute is set down in Table 2. It has not been changed in the last two years, although experimental evaluation of additional drugs has been carried out. The test set is subject to revision as new antibiotics become available and sensitivities of offending microorganisms change.

Dilutions for all antibiotics except carbenicillin and ticarcillin were adjusted to range from 25 mcg/ml to 0.78 mcg/ml. Carbenicillin and ticarcillin were tested in final concentrations between 1250 mcg/ml and 4.5 mcg/ml. The upper level of inhibition which was regarded as placing an organism in the sensitive range was set at 6.25 mcg/ml for gram-positive species (primarily Staphylococci and Streptococci) and

Table 1. Species of Bacteria Tested for Antibiotic MIC: 1976-1981

Species	Year and No. of Strains Tested				
	1976-77	1977-78	1978-79	1979-80	1980-81
<i>Staphylococcus aureus</i>	200	345	245	78	14
<i>Staphylococcus epidermidis</i>	102	35	25	20	20
<i>Streptococcus</i> sp.	44	31	13	10	4
<i>Pseudomonas aeruginosa</i>	184	71	166	84	99
<i>Klebsiella pneumoniae</i>	177	25	15	13	14
<i>Enterobacter cloacae</i>	43	19	0	12	7
<i>Escherichia coli</i>	34	45	16	11	8
<i>Serratia marcescens</i>	14	4	5	0	0
<i>Providencia stuartii</i>	1	0	0	6	38
<i>Proteus mirabilis</i>	38	20	8	6	2

Table 2. Antibiotics Used in MIC Assessment of Sensitivity
1 October 1980 - 30 September 1981

GRAM-POSITIVE ORGANISMS		GRAM-NEGATIVE ORGANISMS	
Antibiotic	Symbol	Antibiotic	Symbol
Gentamicin	G	Gentamicin	G
Tobramycin	To	Tobramycin	To
Oxacillin	Ps	Kanamycin	K
Methicillin	Sc	Amikacin	Ak
Minocin	M	Minocin	M
Vibramycin	Vb	Vibramycin	Vb
Keflin	Kf	Keflin	Kf
Vancomycin	Va	Colistin	Co
Clindamycin	Cl		
Nafcillin	U		

12.5 mcg/ml for gram-negative species. In the case of carbenicillin and ticarcillin, which were tested only against P. aeruginosa, the upper limit for designation as sensitive was set at 312 mcg/ml.

SENSITIVITY OF BURN PATIENT FLORA TO ANTIBIOTICS

Staphylococcus aureus, which has for years ranked high numerically as a cause of bacteremia, fell to an unprecedented low level during this year. For some time the testing routine has included all strains isolated from wound biopsies. This policy ensures minimal delay in making the sensitivity pattern available in those instances in which a positive biopsy connotes active tissue invasion. Nevertheless, with both blood stream and biopsy isolates tested routinely, there were still only 14 strains of S. aureus tested. Twelve of these isolates were from blood stream, one from biopsy, and one from sputum. Table 3 summarizes these results. The sample was small, due to the abrupt decrease in sepsis due to Staphylococci in 1980-1981, but even so, the increase in strains highly sensitive to all categories of antibiotic was striking. The aminoglycosides and vancomycin were completely effective. The semisynthetic penicillins were entirely effective, with only Vibramycin permitting growth of one strain. Vancomycin, long the drug of choice, required 6.25 mcg/ml to suppress all strains tested, although most were suppressed by lower dosage levels. All strains were suppressed by clindamycin.

Comparison of antistaphylococcal activity over the past 8 years (Table 4) places this year's group of strains in marked contrast to earlier years. The entire set tested was inhibited by less than 6.25 mcg/ml of each antibiotic. The earlier fluctuations observed for the aminoglycosides, the methicillin group, and at times even the tetracyclines and clindamycin, were not manifest in this small series. The sensitivity of these isolates differed from those collected in earlier years.

Staphylococcus epidermidis. Attention has been called to the increasing incidence of S. epidermidis in the blood in the early postburn period. There were no instances in this series in which actual sepsis developed, and all but one of these patients yielded only a single positive blood culture. Nor has S. epidermidis burn wound sepsis been seen, so that there appears to be no clinical problem due to this species. One patient, undergoing a prolonged and ultimately fatal course, had three positive blood cultures over a 9-day period. The sensitivity of these isolates from 18 patients is summarized in Table 5. The strains were heterogeneous in response. Although most of them were inhibited by each antibiotic at the 6.25 mcg/ml level, there were isolated resistant to seven of the ten antibiotics tested. The resistant tendency appeared with the same antibiotics in the 1979-1980 collection of strains.

Streptococci. Only four strains of Streptococci, all S. viridans, were recovered in blood culture during this year (Table 6). Percentage of strains is meaningless with such a small number, and the heterogeneity of this population is suggested by the variations in inhibiting levels.

Table 3. Staphylococcus aureus: Cumulative Inhibitory Levels for 14 Strains
1 October 1980 - 30 September 1981

Antibiotic Level mcg/ml	Antibiotic and % Inhibited									
	G	To	Ps	Sc	U	M	Vb	Kf	Va	Cl
> 25.00	100	100	100	100	100	100	100	100	100	100
25.00	100	100	100	100	100	100	100	100	100	100
12.50	100	100	100	100	100	100	100	100	100	100

6.25	100	100	100	100	100	100	100	100	100	100
3.12	92	100	100	100	100	100	100	100	92	100
1.56	78	78	100	100	100	100	92	100	64.2	100
< 0.78	50	35.7	71.4	100	100	100	78	100	0	100

G: Gentamicin; To: Tobramycin; Ps: Oxacillin; Sc: Methicillin; U: Nafcillin; M: Minocin;
Vb: Vibramycin; Kf: Keflin; Va: Vancomycin; Cl: Clindamycin

Table 4. Comparison of Sensitivity of Staphylococcus aureus to Antibiotics
1974 - 1981

Antibiotic	Year and % of Strains Inhibited by 6.25 mcg/ml									
	1974	1975	1976	1977	1978	1979	1980	1981		
Gentamicin	92.2	38.3	50.0	30.6	7.4	17.2	61.0	100		
Tobramycin	88.0	100.0	65.4	16.7	6.2	53.3	100		
Oxacillin	82.6	73.6	70.5	65.1	31.0	75.9	66.2	100		
Methicillin	65.2	21.8	23.5	35.7	77.9	34.6	94.8	100		
Nafcillin	83.3	85.6	49.5	1.8	0.5	0.4	75.6	100		
Minocin	96.0	46.5	92.8	93.9	95.3	96.3	92.3	100		
Vibramycin	78.3	94.2	96.9	42.2	98.0	92.3	100		
Kaflin	90.4	97.2	94.0	97.1	96.9	78.4	79.4	100		
Vancomycin	100.0	100.0	100.0	99.6	98.8	94.8	100		
Clindamycin	95.8	98.0	95.6	97.1	14.4	73.1	94.8	100		

Table 5. Staphylococcus epidermidis: Cumulative Inhibitory Levels for 20 Strains
1 October 1980 - 30 September 1981

Antibiotic Level mcg/ml	Antibiotic and % Inhibited									
	G	To	Ps	Sc	U	M	Vb	Kf	Va	Cl
> 25.00	100	100		100	100	100	100			100
25.00	90	75		95	95	95	90			80
12.50	85	75		95	95	95	90			80
<hr/>										
6.25	85	75	100	95	95	95	90	100	100	80
3.12	85	75	89	95	95	95	90	95	95	80
1.56	85	75	85	95	90	95	90	30	30	80
< 0.78	85	70	63	95	80	95	90	15	15	80

G: Gentamicin; To: Tobramycin; Ps: Oxacillin; Sc: Methicillin; U: Nafcillin; M: Minocin;
Vb: Vibramycin; Kf: Keflin; Va: Vancomycin; Cl: Clindamycin

Table 6. Sensitivity of 4 Strains of Streptococci

Strain	Antibiotic and Inhibiting Level mcg/ml									
	G	To	Ps	Sc	U	M	Vb	Kf	Va	Cl
1	25.00	>25.00	< 0.78	12.59	3.12	12.50	25.00	25.00	3.12	12.50
2	12.50	12.50	6.25	< 0.78	3.12	12.50	6.25	12.50	3.12	>25.00
3	12.50	>25.00	12.50	3.12	12.50	< 0.78	< 0.78	>25.00	< 0.78	< 0.78
4	< 0.78	< 0.78	< 0.78	< 0.78	< 0.78	< 0.78	< 0.78	< 0.78	< 0.78	< 0.78

It was apparent that variations made generalization meaningless, with one exception: all the Streptococci tested were sensitive to vancomycin.

Pseudomonas aeruginosa. This species was numerically predominant as a cause of bacteremia and septicemia in 1980-1981. Ninety-nine of the 208 strains tested were P. aeruginosa. From the chronology of events, it was extremely probable that some of these episodes fell in the category of epidemic outbreaks. Table 7 summarizes the sensitivity patterns observed. There were among these strains 31 from blood culture, 59 from biopsy, five from sputum and four from eye cultures. More than half of the strains were inhibited by 12.5 mcg/ml of gentamicin and by amikacin; the other aminoglycosides were minimally active. Minocin inhibited 92% at the sensitivity level, but Vibramycin inhibited only 78%. Colistin was completely effective in inhibiting Pseudomonas strains, and carbenicillin and ticarcillin were active at the 312 mcg/ml level for almost all strains.

Comparison of sensitivity to the major antibiotics effective against P. aeruginosa over recent years reveals resistance to have developed, only to be replaced by more sensitive populations. Table 8 summarizes this data since 1974. Gentamicin had already decreased in proportion of sensitive strains to 61.8% of strains tested in that year. Sensitive strains decreased steadily to a low of 19.1% in 1976-1977, and the proportion was little changed until 1980-1981, when sensitive strains increased to 64% of the total. Sensitivity to tobramycin was low in all years except 1976-1977, when 61.6% of strains were sensitive. Amikacin, first used in 1976-1977, was highly effective in that year; since that time the sensitive proportion of strains has ranged from 60% to 73%. Minocin was unique in that, during the first two years of testing, few strains were sensitive, but since 1976-1977, sensitive strains have risen from 58.9% to a high of 92% in 1980-1981. The analogue Vibramycin has paralleled Minocin but at a slightly lower level. Colistin has remained highly

Table 7. *Pseudomonas aeruginosa*: Cumulative Inhibitory Levels for 99 Strains
1 October 1980 - 30 September 1981

Antibiotic Level mcg/ml	Antibiotic and % Inhibited of Strains										Conc. mcg/ml	Cb	Tl
	G	To	K	Ak	M	Vb	Kf	Co					
> 25.00	100	100	100	100	100	100	100	100			>1250	100	100
25.00	78	39	13.4	76	100	92.7	1	100			1250	100	96.7

12.50	64	21	10	68	92	78	1	100			625	98.8	96.7
6.25	54	17	4.1	50	57	42	1	98.9			312	98.8	96.7
3.12	36	16	3	29	32	8.2	1	91			-----		
1.56	22	9	2	6	6	0	1	68.3			156	96.5	96.7
< 0.78	4	7	0	1	2	0	0	57.1			78	96.5	96.7
											39	93.6	95.2
											19	93.6	95.2
											9	50	86.2
											4.5	9.0	48.1
											< 4.5	9.0	41.3

Total strains tested	99	99	97	99	99	97	98	98				88	87

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb: Vibramycin; Kf: Keflin;
Co: Colistin; Cb: Carbenicillin; Tl: Ticarcillin

Table 8. Sensitivity of *Pseudomonas aeruginosa* to 3 Aminoglycosides, 2 Tetracyclines, Colistin and 2 Semisynthetic Penicillins

Antibiotic	Year and % of Strains Inhibited by 12.5 mcg/ml					
	1974	1975	1976-77	1978	1979	1980-81
Gentamicin	61.8	40.0	19.1	19.7	25.9	64.0
Tobramycin	18.5	61.6	17.0	4.0	21.0
Amikacin	98.3	60.0	73.5	68.0
Minocin	15.7	16.8	58.9	72.2	86.7	92.0
Vibramycin	20.0	43.6	63.6	60.8	78.0
Colistin	93.3	86.3	89.3	91.3	94.6	100.0
Carbenicillin*	70.8	68.8	58.6	62.0	86.0	96.5
Ticarcillin*	89.4	96.7

* Upper limit to be regarded as sensitive: 156 mcg/ml.

effective during the entire testing period. Carbenicillin inhibited 70.8% of strains in 1974; this level fell to 58.6% in 1976-1977, but has steadily risen since to the current 96.5%. Ticarcillin essentially paralleled these figures for the two years for which data are available.

Klebsiella pneumoniae. This species has for two years been in low incidence; only 14 strains were tested. Twelve of these were from blood, one from sputum and one from biopsy. Eight patients were involved, distributed over the whole year. There was no indication of epidemic involvement, and only two patients had repeated positive blood cultures. The range of sensitivity is shown in Table 9. Sensitivity to aminoglycosides was markedly decreased from strains tested in the previous year. It was especially notable that sensitivity to amikacin fell from 100% in 1979-1980 to 15% in 1980-1981. The tetracyclines were still the most effective antibiotics tested.

Providencia stuartii. This species had for five preceding years been recovered rarely; in two years, no isolates were recovered at all. As late as 1973, a prolonged burn ward epidemic of this species had occurred, and in that year it was the species most commonly recovered in blood cultures, wounds and sputum. In 1979-1980, the species reappeared in patients in the burn ward, and in the current year it became numerically more conspicuous. Thirty-eight strains were tested; of these 19 were from blood, 16 from biopsy and three from sputum. Sixteen patients were the source of these cultures. The previous incursion of Prov. stuartii was marked by an almost complete resistance to the standard battery of antibiotics. This set shows much the same pattern. Table 10 summarizes the results of testing these strains. It is at once evident that only amikacin had any effect on these Providencia strains. Over half would have been designated as sensitive with the 12.5 mcg/ml cut-off designation. It may be added that the colonization of burn patients by this relatively rare opportunist was not accompanied by as marked systemic sepsis among the patients as had previously been the case. Whether this minimally invasive behavior will continue is a question that further monitoring may answer.

Escherichia coli. This and four other species of Enterobacteriaceae were recovered in small numbers this year. However, their sensitivities are reported since there is no assurance, on the basis of previous experience, that any one of these species infrequently encountered in septicemia might not convert to a major epidemic strain. The cumulative sensitivity levels for E. coli are shown in Table 11. The strains were most sensitive to gentamicin, to Minocin and to colistin. Keflin was effective at the 50% range. The variations in sensitivity indicate the heterogeneous origin of the strains.

Enterobacter cloacae. This enteric species is the last of consequence recovered from biopsies (6) and blood culture (1) during this period. Four patients yielded this species. Since the organism had a record of causing severe epidemic episodes of sepsis on the burn ward in

Table 9. Klebsiella pneumoniae: Cumulative Inhibitory Levels for 14 Strains
1 October 1980 - 30 September 1981.

Antibiotic Level mcg/ml	Antibiotic and % Inhibited							
	G	To	K	Ak	M	Vb	Kf	Co
> 25.0	100	100	100	100	100	100	100	100
25.0	85	14.2	14.2	15	75	64	64	100
12.5	85	14.2	14.2	15	75	57	42	100
6.25	85	14.2	14.2	15	66	57	28	92
3.12	71	14.2	14.2	15	50	57	28	92
1.56	50	14.2	7.1	0	16	14.2	0	92
< 0.78	7.1	7.1	7.1	0	0	0	0	35

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb:
Vibramycin; Kf: Keflin; Co: Colistin

Table 10. Providencia stuartii: Cumulative Inhibitory Levels for 38 Strains
1 October 1980 - 30 September 1981

Antibiotic Level mcg/ml	Antibiotic and % Inhibited							
	G	To	K	Ak	M	Vb	Kf	Co
> 25.00	100	100	100	100	100	100	100	100
25.00	0	0	2.6	68.4	10.5	0	0	0

12.50	0	0	0	57.9	0	0	0	0
6.25	0	0	0	2.6	0	0	0	0
3.12	0	0	0	0	0	0	0	0
1.56	0	0	0	0	0	0	0	0
< 0.78	0	0	0	0	0	0	0	0

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb:
Vibramycin; Kf: Keflin; Co: Colistin

Table 11. *Escherichia coli*: Cumulative Inhibitory Levels for 8 Strains from Blood and Tissue Biopsies: 1 October 1980 - 30 September 1981

Antibiotic Level mcg/ml	Antibiotic and % Inhibited							
	G	To	K	Ak	M	Vb	Kf	Co
> 25.00		100	100	100		100	100	
25.00	100	87	62.5	75	100	62.5	50	

12.50	87	62.5	62.5	75	87	50	50	
6.25	87	37	62.5	25	75	37	25	
3.12	50	25	25	12.5	62.5	37	12.5	100
1.56	0	0	0	12.5	50	37	0	87
< 0.78	0	0	0	12.5	50	25	0	62.5

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Co: Colistin

past years, the sensitivity of this small collection is set down in Table 12. Five of the seven strains were relatively sensitive to three of four aminoglycosides. The strains varied in response to tetracyclines, were resistant to Keflin and sensitive to colistin. These values were much more in the sensitive range than those observed at the end of the last epidemic episode of Enterobacter cloacae. In that collection, in 1977, strains were essentially resistant to gentamicin, Kantrex, and Keflin. They were highly sensitive to Minocin and to Vibramycin, and, in the first year of use, to amikacin.

The remaining species were recovered in inconsequential numbers and their sensitivity values cannot represent a significant sample of these bacteria. The minor isolates include Enterobacter aerogenes (1), Citrobacter diversus (1), Aeromonas hydrophila (1), Acinetobacter lwoffii (1), Citrobacter amalonaticus (1) and Proteus mirabilis (2).

DISCUSSION

The epidemiologic phenomenon of especial note during 1980-1981 was the reduction of S. aureus to a minor role in the cause of sepsis in severely burned patients. The only continuing epidemic species was P. aeruginosa. Formerly important enteric species, Klebsiella pneumoniae and Enterobacter cloacae, were not conspicuous at any time. The reappearance of Prov. stuartii as a cause of septicemia and tissue invasion was the other new phenomenon. Although not as numerous as in the epidemic years, they were sufficiently frequent in sepsis to be of concern. The sensitivity of these isolates was disturbingly low; only amikacin showed significant activity against this species. Its further course in the burn ward merits close surveillance.

The drop in incidence of S. aureus makes current assessment of sensitivity to antibiotics, particularly to the semisynthetic penicillins, impossible. Such observations have, for the present, stopped with the 1979-1980 reporting period.

PRESENTATIONS/PUBLICATIONS - None

Table 12. *Enterobacter cloacae*: Cumulative Inhibitory Levels for 7 Strains
from Biopsy and Blood: 1 October 1980 - 30 September 1981

Antibiotic Level mcg/ml	Antibiotic and % Inhibited						
	G	To	K	Ak	M	Vb	Co
> 25.00	100	100	100	100	100	100	100
25.00	70	70	57	70	85.7	70	85.7

12.50	70	70	57	70	85.7	70	85.7
6.25	70	70	57	70	70	70	85.7
3.12	70	70	57	70	57	42	85.7
1.56	70	70	42	70	0	14	85.7
< 0.78	57	14	14	70	0	14	70

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb:
Vibramycin; Kf: Neflin; Co: Colistin

ANNUAL PROGRESS REPORT

PRO. IO. 3M161102BS10-00, BASIC RESEARCH
REP. TLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- SEROLOGIC TYPES OF
PSEUDOMONAS AERUGINOSA FOUND IN BURNED SOLDIERS

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Investigators:

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

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Serotyping of Pseudomonas aeruginosa was carried out on 608 isolates from 79 patients. Specimens were primarily sputum and wound cultures. Serotypes antigenically distinctive totalled 29, a marked increase from the previous year. For the first time in this series of observations, a monotype epidemic was shown to have occupied the Institute of Surgical Research burn wards for the entire year; 85.8% of all isolates typed were type 15. The remainder of strains were scattered over 28 types, with only type 11 showing a transient numerical predominance. The typing approach is valid, valuable and relatively effective. There are discrepancies in the typing sera that merit further effort to perfect the system.

Pseudomonas
Burns
Serotype
Infection
Epidemic
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS
WITH THERMAL INJURY -- SEROLOGIC TYPES OF PSEUDOMONAS AERUGINOSA
FOUND IN BURNED SOLDIERS

Pseudomonas aeruginosa has remained a prominent feature of the burn flora in patients in this Institute for more than 20 years. The total of patients harboring this important burn pathogen can vary over periods of a year or more, as can the incidence of sepsis due to Pseudomonas, but the organism has not been reduced significantly in incidence over any period of time. Burn ward epidemics in which one strain of this organism predominated in the burn flora have been repeatedly documented. Recognition of a nosocomial epidemic is essential if there are to be any attempts at control of the episode.

The P. aeruginosa population in the Institute of Surgical Research wards has been continuously monitored at the basic level of differentiation of individual isolates since 1960. Phage typing, using the ISR typing set developed here, is the most precise and accurate technic available, but since 1976 it has not been possible to sustain this time-consuming and exacting procedure. The serotyping technic, using the 17-factor set proposed by an international committee, has shown a degree of specificity and sensitivity that permits a useful differentiation of strains, although the somatic antigen approach leaves groups of strains which could be separated by more sensitive technics.

As was described in the 1979-1980 report, typing was facilitated by dividing the 17-type set into two sub-sets. The first set, containing factors 3, 4, 6, 8, 9, 10, 11, 15 and 16, is used on all strains typed. Experience has shown that over 90% of typable isolates tested will react with one or more of these series. Negative reactors are tested with the remaining eight factors. In addition, strains from each group of isolates are checked with these second-set sera, to ensure that additional factors in the organism are not overlooked. Most typing was done on live-cell suspensions. Autoclaved suspensions added a small number of typed strains that would otherwise have been missed. However, in no instance would an epidemic pattern have been altered had duplicate typings been done. The autoclaved suspension is a useful adjunct in complete typing, but it is not a means of characterizing a large number of otherwise untypable strains.

TYPES OF PSEUDOMONAS AERUGINOSA OBSERVED

Five hundred and ninety-seven of 608 strains of P. aeruginosa were typable during this observation period. The strains were collected from 79 patients. The distribution of types recovered is shown in Table 1. There were 29 different types recognized, but only one, type 15, was present in a large preponderance over all others. Although in other years smaller groups of strains were present briefly in epidemic proportion, this cannot be detected in the 1980-1981 collection. The types recovered in the years since 1976 show the successive changes that have occurred. Since 1978-1979, the number of types recognized has fluctuated between 25 and 33.

Table 1. Serotypes of Strains of Pseudomonas aeruginosa from 79 Burn Patients, 1980-1981

Type	No. of Strains
1,3,5,6,8,9,10,13	1
3	2
3,8,16	1
4	7
4,10,11,15	1
6	5
6,8,9,10,13	2
6,9, 10	1
6,9,10,13	8
6,9,13	3
6,10,13	1
6,11	1
6,13	3
6,16	1
7	1
8	1
8,9,11,15	1
8,9,15	2
8,11	1
9,10	3
10	1
10,15	1
11	18
11,15	1
11,16	1
12	1
15	512
15,16	14
16	2
Non-typable (no reaction)	11
Total strains	608
Total typed	597
Total types	29

A scanning of Table 2 shows, by number of strains of a given type collected, the epidemic pattern of this organism in successive years. Type 4 was prominent in epidemic episodes in each year since 1976, until the present accounting. No epidemic episodes due to type 4 occurred in 1980-1981. Type 4,9,10 incited one outbreak in 1976-1977. It has not returned in significant numbers since. Type 6 also incited an outbreak in 1976-1977; it has been found in the past three years, but never in a pattern of epidemic transmission. Type 8 occasioned an outbreak in early 1978; it was found in small numbers in succeeding years, but not in any degree of consequence. Type 10, or its factor, appears in many combinations; there are 23 type patterns that include it. But only once, in 1979-1980, did it reach an epidemic scale. Type 11 has in each of the past four years incited one or more epidemic outbreaks, and in 1980-1981, the pattern was that of an epidemic. However, none of these episodes was long-lived.

The single recurrent epidemic type since 1976 has been type 15. There are certainly sub-types within this group, but the vast majority of isolates were pure type 15. In 1980-1981, it was, with the exception of one outbreak caused by a type 11 strain, the only type of P. aeruginosa present in an epidemic pattern. The fact that, despite continuous seeding and reseeded of the burn patients with this strain, there was no marked rise in the incidence of *Pseudomonas* sepsis, suggests that it is a strain of moderate or even minimal virulence.

The appearance of new types is an ongoing phenomenon. In 1976-1977, two types were recovered which have not been seen since. Six types were unique to 1977-1978, and three in 1978-1979. In 1979-1980, there were 13 strains of types seen only in that year. In 1980-1981, the preponderance of type 15 would lead one to expect fewer new types to have had the opportunity to emerge. However, 12 strains of types not previously recorded were seen in this year. They were primarily types including factors 6 and 9. These types, unique as yet to a single year, are presented in Table 3. Inspection of this data shows that the major part of the separate types in the past two years have been strains linked in combinations of factors that are evidently labile. Thus type 4,9, type 4,9,10,11 and type 4,10,11 might each be recovered from a series of cultures on a single patient. This situation was the case in 1980-1981, where the variations on a type 6,9,10,13 made up multiple different types. However there is no valid basis for discarding these antigenically determined identities. The multivalent types shown were each checked at least in triplicate on successive subcultures. The identities could be determined by repeat culture. The antigenic system embodied in the International Typing Set does indeed merit further analysis and possibly improvement in the antigens selected and their monovalent identities. However, at present we can no more discard a plethora of types seldom encountered than we can impugn the validity of the monotype determinant of the predominant type, 15, in 1980-1981.

The possibility that some types of P. aeruginosa are unusually capable of inciting sepsis has been a basic reason for ongoing efforts

Table 2. Comparison of Incidence of Serotypes of Pseudomonas aeruginosa from Burn Patients, 1976-1981

Type	Year & No. of Isolates				
	1976-77	1977-78	1978-79	1979-80	19
1				1	
1,2,3,4,9,10		7			
1,3,5,6,8,9,10,13					
1,9	2			1	
2,3,6,15	3				
3		9	2		
3,4,9,10		1			
3,8,9,14		2			
3,8,16					
3,10			2	2	
3,15			2		
4	110	183	42	102	
4,6,9,10,16				2	
4,8,9,11,12,14		2			
4,9				1	
4,9,10	20	12		2	
4,9,10,11				11	
4,9,11				1	
4,10			21	20	
4,10,11				1	
4,10,11,15					
4,10,13		2			
4,10,15			1		
4,10,16				1	
4,11		1	1	3	
4,11,15,16			1		
4,15		2	2		
4,16				2	
5		1	1		
5,8,16				1	
6	38		5	11	
6,8,9,10,13					
6,9,10					
6,9,10,13					
6,9,13					
6,10,13					
6,11					
6,13					
6,16				3	
7		3			
8		17	1	3	
8,9		2			
8,9,11,15					
8,9,15					
8,11			1		

Table 2. Comparison of Incidence of Serotypes of Pseudomonas aeruginosa from Burn Patients, 1976-1981 (Continued)

Type	Year & No. of Isolates				
	1976-77	1977-78	1978-79	1979-80	1980-81
8,11,15,16				2	
8,12		1	1		
8,15,16				1	
9	2	2		1	
9,10	2	10	2	3	3
9,16				1	
10	3	16	6	31	1
10,11,15				1	
10,15			1		1
10,15,16			1	1	
10,16				1	
11		35	89	69	18
11,15			3	1	1
11,15,16			1		
11,16				4	1
12		1	2		1
14	7				
15	239	119	158	171	512
15,16			1	9	14
16		4	11	6	2
Non-typable	3	21	43	37	11
Total strains	429	453	401	507	608

Table 3. Serotypes of Pseudomonas aeruginosa Which Have Appeared in Only
a Single Year, 1976-1981

1976-77	1977-78	1978-79	1979-80	1980-81
2,3,6,15 14	1,2,3,4,9,10 3,4,9,10 3,8,9,14 4,8,9,11,12,14 4,10,13 8,9	3,15 4,10,15 11,15,16	1 4,6,9,10,16 4,9 4,9,10,11 4,9,11 4,10,11 4,10,16 4,16 5,8,16 8,11,15,16 8,15,16 10,11,15 10,16	1,3,5,6,8,9, 10,13 3,8,16 4,10,11,15 6,8,9,10,13 6,9,10 6,9,10,13 6,9,13 6,10,13 6,11 6,13 8,9,11,15 8,9,15

at developing technics for differentiation of strains. A review of the sources of individual strains in relation to the site of recovery is appropriate to address this question. Types recovered from blood, wound (both surface and biopsy cultures), sputum and autopsy cultures are summarized in Table 4. Only one type, 15, was recovered from the blood of more than one patient. There were five types recovered from blood. It is of interest to note that one of these, type 6, was recovered only once, from a patient whose wound and sputum did not harbor type 6, but instead the predominant type 15. There were 14 types recovered from wounds, nine from sputum and six from autopsy tissues. The smaller numbers of strains of each type recovered in this year reflects the overwhelming preponderance of type 15 in the burn ward. It is of interest to note that type 15 was described, in the 1979-1980 report as "...present, but not a major feature of the 1979 epidemic period. However it rose to a striking peak in 1980 from May through September." This comment is consistent with subsequent events; a virtual monotype epidemic with P. aeruginosa characterized the 1980-1981 period. The types which have caused micro-epidemics in previous years were represented in the 1980-1981 collection. But types 4, 6, 8, and 10 which have caused outbreaks in burn wards in the past did not do so this year. A small episode of type 11 infection occurred but by criteria set up for assessing epidemics, it would not qualify for that category. Thus, in 1980-1981, there was only one type that qualified as an epidemic strain: type 15.

DISCUSSION

Monitoring P. aeruginosa in the burn ward by use of serotyping was again shown to be an effective approach. The epidemiologic pattern was one not previously seen; only one strain, by serotype, was present in significant numbers in the ward, and it was almost totally predominant. Again, this information would be vital to a specific attempt to decontaminate the burn ward and its population, and to break this chain of cross-infection. Whether this approach is a valid one is an entirely separate question. Meanwhile, ongoing monitoring will tell us whether and when changes occur in the *Pseudomonas* flora of the burn ward. Correlations with virulence of offending strain, response to chemotherapeutic agents, and tendency to produce invasive infection can be made by this approach.

PRESENTATIONS/PUBLICATIONS - None.

Table 4. Serotypes of Pseudomonas aeruginosa from Blood, Sputum, Wound and Autopsy Tissues of Burn Patients, 1980-1981

Serotype	Blood	Wound	Sputum	Autopsy
1,3,5,6,8,9,10,13		1		
3		2		
3,8,16		1		
4		4	2	
4,10,11,15			1	
6	1			
6,8,9,10,15				1
6,9,10		1		
6,9,10,13		7	1	
6,9,13		1		
6,11		1		
6,13	1	2		
6,16			1	
7			1	
8				1
8,9,11,15				1
8,9,15				3
9,10	1	2		
10		1		
11		10	4	
11,16			1	
15	19	229	146	60
15,16	1		2	
16		1		2

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

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WOUND INFECTION: BACTERIAL FLORA OF BURN WOUNDS OF
MILITARY PERSONNEL RECEIVING SULFAMYLON OR SILVER
SULFADIAZINE TREATMENT

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The cause of bacterial infection in burns can be assessed by detailed analysis of burn patient flora on a periodic basis. Such monitoring has revealed the existence of an extensive list of species, some capable of establishing prolonged epidemics in burn wards, while others are capable of short-term epidemics and many cannot apparently propagate even a transient epidemic episode. During the 1980-1981 period, Staphylococcus aureus diminished to a new low in incidence in burn wards. No enteric species established severe epidemics of sepsis, but Providencia stuartii increased in incidence after a prolonged absence, to reach numbers exceeded only by Pseudomonas aeruginosa. Only P. aeruginosa incited sepsis in burn patients on an epidemic scale.

Burns
Staphylococci
Pseudomonas
Providencia
Sepsis
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH
THERMAL INJURY -- PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL
FLORA OF BURN WOUNDS OF MILITARY PERSONNEL RECEIVING SULFAMYLON OR
SILVER SULFADIAZINE TREATMENT

Infection in burn wounds, together with secondary systemic infection, is the major cause of morbidity and death in burn patients. For almost two decades, intensive effort has been directed toward improved control of such infection, and there have been significant strides in improving infection control. However, because of the opportunistic nature of such infections, no specific human pathogen has played a dominant causative role, and the burn treatment team has been continually confronted with a changing population of opportunistic pathogens. These strains have in common a propensity for giving rise to antibiotic-resistant variants, which predictably minimizes the scope of antimicrobial therapy. Species of Enterobacteriaceae, Pseudomonas aeruginosa, and Staphylococcus aureus have been the principal offenders in the past 15 years. In the previous report (1979-1980), it was pointed out that the incidence of sepsis appeared to have declined during that year. The number of patients from whom blood cultures were collected fell, and the number who exhibited bacteremia declined markedly from that seen in previous years. During 1980-1981, this trend continued. Its existence may be validated, but an explanation for this important trend has not been derived.

Precise identification of bacterial isolates from burn patients has been emphasized; although this practice will exhibit large numbers of species occurring only in small numbers, it is fundamentally the only way in which the capability of species for burn patient colonization can be determined.

ANTEMORTEM BACTERIOLOGY OF BURN PATIENTS, 1980-1981

Bacterial and yeast species recovered during this 12-month period are listed in Table 1. The anatomic source of each strain and the number of isolates recovered are presented.

There were 194 patients during this period from whom at least one culture was positive. Patients on whom no cultures were done are omitted from consideration. Of all patients cultured, 52% had a wound culture taken. Contact plate samples were taken on 73% of all patients cultured, blood cultures from 90%, sputum cultures from 52%, urine cultures from 73%, and biopsy samples from 24% of all patients cultured. Wound cultures by swab and contact plate cultures might have been expected to yield comparable recoveries. This was not the case: Staphylococcus epidermidis was recovered twice as often by contact plate as by wound swab. Providencia stuartii was also recovered far more often by contact plate than by swab. The high level of negative wound swabs reported has typified wound culture results in the past three years. A similar low rate of positive wound swabs was seen in 1961 and 1962. At that time, strenuous

Table 1. Antemortem Bacteriology in 194 Burn Patients, 1 October 1980 - 30 September 1981

Organism	Source and Number of Isolates									
	Wound Surface			Catheter Tips						
	Swab	CP	Blood	Sputum	Urine	IV	Foley	Biopsy	Grafts	Stool Total
Staph. aureus	38	29	11	224	7	5	2	8	3	5
Staph. epidermidis	21	45	18	58	14	5	7	0	0	0
Micrococcus sp.	0	0	0	1	0	0	0	0	0	1
Gp A Streptococcus	1	0	0	7	0	0	0	1	0	0
Gp B Streptococcus	0	0	0	3	0	0	0	0	0	3
Gp C Streptococcus	0	0	0	2	0	0	0	0	0	2
Strep. viridans	22	10	3	224	4	1	2	0	0	0
Non-hemol. Strep. not Gp D	7	6	2	63	11	0	1	0	1	1
Gp D Strep. not an Entero.	4	3	0	36	3	0	1	3	0	3
Gp D Enterococcus	5	8	2	18	12	0	3	5	0	2
Streptococcus pneumoniae	4	0	0	21	0	0	0	0	0	0
Beta-hemol. Strep. not Gp A, B or D	1	0	0	42	0	0	0	1	0	0
Enterobacter aerogenes	9	7	1	37	1	0	0	0	0	0
Enterobacter cloacae	2	4	0	23	2	2	1	8	1	0
Enterobacter agglomerans	0	0	0	7	2	0	0	1	3	0
Klebsiella pneumoniae	14	18	10	110	29	9	5	3	5	3
Klebsiella oxytoca	8	2	0	27	1	0	0	1	3	1
Klebsiella ozaenae	2	0	0	2	0	0	0	0	7	1
Proteus mirabilis	8	17	1	33	13	1	3	1	0	0
Morganella morganii	1	0	0	3	0	1	0	2	0	0
Serratia marcescens	0	0	0	7	0	0	0	0	0	7
Providencia stuartii	41	178	31	133	33	18	7	28	5	1
Escherichia coli	31	15	4	52	69	2	8	2	12	9
Hafnia alvei	0	0	0	3	0	0	0	0	0	0
Aeromonas hydrophila	1	3	3	1	0	0	0	0	1	0
Citrobacter diversus	4	1	1	10	2	0	0	0	0	2
Citrobacter freundii	2	0	0	1	4	1	0	0	2	0
Citrobacter amalonaticus	0	0	0	0	0	0	0	1	0	0
Acinetobacter anitratus	0	3	0	7	1	0	0	1	8	0
Acinetobacter lwoffii	2	2	0	9	0	0	0	2	5	0
Haemophilus aphrophilus	0	0	1	0	0	0	0	0	0	1

Table 1. Antemortem Bacteriology in 194 Burn Patients, 1 October 1980 - 30 September 1981 (continued)

Organism	Source and Number of Isolates										Total		
	Wound Surface			Blood	Sputum	Urine	Catheter Tips			Biopsy		Grafts	Stool
	Swab	CP					IV	Foley					
<i>Pseudomonas aeruginosa</i>	130	179	43	249	47	10	7	64	20	0	749		
<i>Pseudomonas fluorescens</i>	0	3	0	1	0	0	0	0	42	0	46		
<i>Pseudomonas putida</i>	1	4	2	3	0	0	0	0	111	0	121		
<i>Pseudomonas maltophilia</i>	0	0	0	0	1	0	0	0	2	0	3		
<i>Pseudomonas cepacia</i>	0	2	0	1	1	0	0	2	3	0	9		
<i>Pseudomonas stutzeri</i>	0	0	0	0	0	0	0	0	6	0	6		
<i>Pseudomonas alcaligenes</i>	0	1	0	0	0	0	0	0	0	0	1		
<i>Pseudomonas sp.</i>	0	1	0	4	0	0	0	0	88*	0	93		
<i>Alcaligenes faecalis</i>	0	2	0	0	0	0	0	1	1	0	4		
Gp 2K-1	0	1	0	0	0	0	0	0	0	0	1		
<i>Flavobacterium sp.</i>	0	1	0	0	0	0	0	0	0	0	1		
<i>Achromobacter xylosoxidans</i>	0	0	0	1	0	0	0	0	0	0	1		
<i>Corynebacterium sp.</i>	1	2	1	3	0	0	0	0	2	0	9		
<i>Neisseria sp.</i>	10	3	0	51	0	0	0	0	0	0	64		
<i>Bacillus sp.</i>	5	88	2	6	2	2	0	1	4	0	110		
<i>Candida albicans</i>	1	7	1	28	41	4	1	0	1	1	85		
<i>Candida rugosa</i>	2	10	0	0	7	2	1	5	0	0	27		
<i>Candida tropicalis</i>	0	3	0	4	12	0	1	5	0	0	25		
<i>Candida zeylanoides</i>	0	0	0	0	0	0	0	0	1	0	1		
<i>Candida krusei</i>	0	0	0	0	16	0	0	0	0	0	16		
<i>Trichosporon beigelli</i>	0	1	0	2	2	0	0	1	0	0	6		
<i>Torulopsis glabrata</i>	0	0	0	0	2	0	0	0	0	0	2		
Yeast-like organism	0	2	1	1	0	1	0	0	0	0	5		
<i>Microsporium sp.</i>	0	0	0	0	0	0	0	1	0	0	1		
<i>Aspergillus sp.</i>	0	0	0	0	0	0	0	10	0	0	10		
<i>Alternaria sp.</i>	0	0	0	0	0	0	0	4	0	0	4		
<i>Fusarium sp.</i>	0	0	1	0	0	0	0	3	0	0	4		
<i>Mycelia sterilia</i>	0	0	0	0	0	0	0	3	0	0	3		
Number isolates:	378	661	139	1518	339	64	50	168	337	29	3683		
Number of specimens:	382	659	1499	594	830	154	61	212	450	11	4852		
Number of patients:	102	142	176	101	142	52	30	47	48	5			

* Speciation on graft isolates was discontinued in June 1981 after review of results indicated these strains were not playing a role in clinical illness and constituted an unjustifiable expense if they were to be classified in detail.

indoctrination in careful wound swab sampling technic was undertaken, and the proportion of negative results from wound swabs dropped from over 50% of samples to 5% of samples. At intervals during the past two decades, a rise in negative results from wound swab cultures has prompted renewed emphasis on correct sampling technic. In each instance, the number of negative wound swabs has decreased sharply. It is probable that the present high incidence of negative wound swabs reflects errors in sampling technics. Failure to clean surfaces of antimicrobial agents, use of dry swabs instead of moistened swabs, swabbing of inappropriate areas of the wound and too timid an approach to swabbing the wound are prominent factors in failure to recover bacteria from open burn wounds. An additional manipulative error in the results of wound culturing is the high level of Bacillus sp. reported from contact plates. The preparation of a contact plate culture as conducted at the Institute of Surgical Research involves trimming the gauze "handles" which permit the agar sheet to be applied to the wound. Transfer of contaminating Bacillus sp. from the person doing the sampling can readily occur, and such growth is reported as part of the wound flora. In establishing the contact plate technic in the 1960s, the importance of aseptic technic in trimming the gauze was recognized. Repeated episodes of Bacillus sp. recoveries not matched by wound swab, biopsy, or autopsy results indicate that this error is introducing an artifact into the results of contact plate sampling of wounds. As with swab cultures, negative contact plates have been recently reported at a far higher level than was ever observed during the period of 1963 to 1970, when the senior author (RBL) collected plates personally. No negative culture was ever observed out of over 6,000 cultures taken during that time.

The most common species recovered from wounds were S. aureus, Strep. viridans, Kleb. pneumoniae, Prov. stuartii and P. aeruginosa. In blood cultures, a notable change from recent years was a fall in the incidence of S. aureus. Providencia stuartii reappeared in blood cultures in significant numbers this year for the first time since 1973. In order of frequency of occurrence, the most common species recovered were P. aeruginosa (749), Prov. stuartii (475), S. aureus (332), Strep. viridans (266), Kleb. pneumoniae (206), E. coli (204) and S. epidermidis (168). Candida albicans was the most common yeast species, in a population of decreasing numerical importance.

There were 46 different species of bacteria recovered. The annual incidence has remained close to this value for several years.

Obviously total strains collected do not reflect the relative importance of species as infecting or colonizing agents. The number of patients positive for a given species reflects more precisely that organism's potential for spread, colonization and infection. A resume of patients colonized or invaded by the major species recovered is shown in Table 2. The significant species included S. aureus, S. epidermidis, Kleb. pneumoniae, Prov. stuartii, P. aeruginosa and E. coli. Patients were most often colonized and invaded by P. aeruginosa, as was evident from the number positive in wound, blood, sputum and biopsy. Prov. stuartii, which was totally absent from the burn ward for over

1 October 1960 - 30 September 1961

Organism	Source and Number of Patients Positive in Culture							
	Wound Surface		Catheter Tips					
	Swab	CP	Blood	Sputum	Urine	IV	Foley	Biopsy Grafts
<i>Staphylococcus aureus</i>	24	21	9	56	6	4	2	5
<i>Staphylococcus epidermidis</i>	18	37	17	37	11	4	7	0
<i>Klebsiella pneumoniae</i>	11	11	6	33	18	5	5	3
<i>Providencia stuartii</i>	20	49	11	30	14	9	7	12
<i>Pseudomonas aeruginosa</i>	48	64	19	52	19	9	6	22
<i>Escherichia coli</i>	25	11	4	24	22	2	8	2
Total no. patients sampled	102	142	176	101	142	52	30	47
								48

two years until its gradual reappearance in 1980, was found in approximately as many patients as was P. aeruginosa in 1980-1981.

As a more effective way of showing the degree of involvement of burn patients with bacteria in this year, the percentage of patients cultured at each body site who were positive for the major species is shown in Table 3. In terms of proportion of patients involved, the major species were S. aureus, S. epidermidis, Kleb. pneumoniae, Prov. stuartii, and P. aeruginosa. In comparison with 1979-1980, the most noticeable changes were the increase in infections due to S. epidermidis and the reappearance of Prov. stuartii. Species formerly seen in significant numbers but absent this year included Enterobacter cloacae which rather abruptly fell to an inconsequential incidence.

Table 3. Percentage of Patients Cultured and Positive for Major Bacterial Species at Significant Culture Sites
1 October 1980 - 30 September 1981

Organism	Source and % of Cultured Patients Positive			
	Wound	Biopsy	Blood	Sputum
Staph. aureus	18	11	5	55
Staph. epidermidis	23	0	10	37
Klebsiella pneumoniae	9	6	3	33
Proteus mirabilis	8	2	0.6	8
Providencia stuartii	28	26	6	30
Escherichia coli	15	4	2	24
Pseudomonas aeruginosa	46	47	11	51

BACTERIOLOGY OF BURN WOUNDS

Monitoring of burn wound flora is a widely accepted practice, although the value of the information produced by such cultures is not clear in terms of therapy of the burn. Topical therapy, surgical procedures and severity of the burn all exert striking effects on burn wound flora. Wounds in the Institute of Surgical Research are in most instances exposed to both Sulfamylon and silver sulfadiazine, and these agents undoubtedly affect the nature of the bacterial flora of the burn. No topical therapy will maintain a burn in the sterile state.

The microbial flora of the burn wounds of 142 patients cultured in this period is shown in Table 4. Numerically significant species included S. aureus, S. epidermidis, Strep. viridans, Prov. stuartii, E. coli and P. aeruginosa. No other species reached even 10 percent of the patients cultured. In view of previous wound flora events, however, even some

Table 4. Burn Wound Surface Flora in 142 Patients
1 October 1980 - 30 September 1981

Organism	No. of Strains	No. of Patients Positive	% of Cultured Patients Positive
Staph. aureus	67	24	17
Staph. epidermidis	66	37	26
Streptococcus Gp A	1	1	0.7
Strep. viridans	32	18	13
Non-hemol. Strep. not Gp D	13	7	5
Gp D Strep. not Enterococcus	7	4	3
Strep. Gp D Enterococcus	12	8	6
Strep. pneumoniae	4	4	3
Enterobacter cloacae	6	4	3
Enterobacter aerogenes	16	6	4
Klebsiella pneumoniae	32	11	8
Klebsiella oxytoca	10	5	4
Klebsiella ozaenae	2	2	1
Proteus mirabilis	25	14	9
Providencia stuartii	219	49	35
Escherichia coli	46	25	18
Aeromonas hydrophila	4	3	2
Citrobacter diversus	5	4	3
Citrobacter freundii	2	2	1
Acinetobacter anitratus	3	3	2
Acinetobacter lwoffii	4	2	1
Pseudomonas aeruginosa	309	64	45
Candida albicans	8	5	4
Candida rugosa	12	6	4
Candida tropicalis	3	2	1

of the numerically inconsequential species are of interest. Enterobacter cloacae and Kleb. pneumoniae had in previous years had long periods in which they were a major factor in wound colonization and infection. In this year, they were inconsequential. Similarly, the Acinetobacter genus has been involved in intense episodes of wound infection, but not in this observing period. A species noteworthy because of its total absence was Serratia marcescens. This opportunistic invader has previously been seen at least in small numbers, but not this time. There were only 22 species of bacteria recovered from wounds; in the previous year there had been 31.

Group A streptococci, because of their extremely destructive capability when they infect a skin graft, have long been monitored with particular care, since the presence of this organism in a burn wound could precipitate disastrous infections. After several years in which small numbers of strains were recovered from wounds, the species had been absent from the burn ward since 1978. As is seen here, one wound strain was recovered this year. The species remains a relative curiosity in burn wounds.

RESPIRATORY TRACT FLORA IN BURN PATIENTS

The flora of the respiratory tract continues to constitute a population of intrinsic interest, since control of pneumonia has always been a significant problem in the management of patients with severe burns. In Table 5, the bacterial species in sputum or Luken's tube aspirates from 101 patients are summarized. Numerically, the most frequent species was Strep. viridans, from 72% of patients cultured. However, no significant degree of pulmonary pathology was related to the strain. It behaved as an innocuous part of the normal flora. S. aureus was recovered from respiratory secretions of over half of the patients, as was P. aeruginosa. Kleb. pneumoniae (33%) was the most common enteric species in sputum, closely followed by Prov. stuartii (30%). Other species which have in the past played a prominent role in pulmonary involvement, especially Entero. cloacae, were not common in this year.

SEPTICEMIA IN BURN PATIENTS

Systemic sepsis is the most serious aspect of bacterial infection in burn patients, and this aspect of burn bacteriology merits careful scrutiny. Other sites of colonization may or may not signify an active infectious process, but bacteria in the blood stream are probably the most meaningful indication of invasive involvement with a microorganism. In 1980-1981, 176 patients had blood cultures drawn. These were emphatically not all from seriously ill patients. A belief that early blood cultures may reveal unsuspected sepsis has prompted the taking of large numbers of cultures on patients who exhibit few clinical indications of bacteremia. However, significant information regarding the septicemic potential of colonizing strains can be adduced from the results of these blood cultures. Table 6 shows the total of 16 strains of bacteria, together with two yeasts and one fungus recovered in blood cultures. The most important

Table 5. Principal Species of Bacteria Recovered from Respiratory Tract of 101 Patients, 1 October 1980 - 30 September 1981

Organism	No. of Isolates	No. of Patients Positive	% of Cultured Patients Positive
Staph. aureus	224	56	55
Staph. epidermidis	58	37	37
Strep. viridans	224	73	72
Non-hemol. non-Gp D Strep.	63	29	29
Enterococcus Gp D	18	8	8
Non-Gp D Enterococcus	36	19	19
Strep. pneumoniae	21	13	13
Gp A Strep.	7	2	2
Beta-hemol. Strep. not Gp A, B or D	2	2	2
Enterobacter aerogenes	37	15	15
Enterobacter cloacae	23	16	16
Klebsiella pneumoniae	110	33	33
Klebsiella oxytoca	27	11	11
Proteus mirabilis	33	8	8
Providencia stuartii	133	30	30
Escherichia coli	52	24	24
Citrobacter diversus	10	4	4
Pseudomonas aeruginosa	249	52	51
Neisseria sp.	51	28	28
Candida albicans	28	15	15

species from the standpoint of number of patients who yielded them in blood culture were P. aeruginosa, Prov. stuartii and Kleb. p. among gram-negative bacilli, and S. aureus and S. epidermidis as positive flora. The entire picture of sepsis in burn patients has to have diminished sharply, in comparison to the numbers seen five years ago, in 1976-1977. Of course, the number of seriously burned patients was not necessarily comparable between this year and earlier years among patients admitted since October 1979 to the present, there has to have been a lessening incidence of septicemia during the course of their illness. The numerically high incidence of S. epidermidis from blood culture was commented upon in the previous annual report -- was never recovered more than once -- usually early in the patient's course -- and no clinical sepsis due to this species has been reported. The diverse population of species recovered from one or two patients suggests a broad capability of such opportunistic contaminants to enter the blood stream, but no indication of epidemic pattern could be recognized.

In previous years, as many as half of the patients exhibit septicemia displayed multiple invasion by more than one bacterial species. The incidence of such mixed infections fell significantly in 1979-1980; 22 out of 217 patients who had blood cultures drawn more than one species of organism recovered on successive episodes. This was itself a drop from previous years, but in 1980-1981, the incidence of mixed infections fell to a negligible figure. Table 1 shows the patients who had more than one species recovered from blood cultures. There were only four such patients, and two of these had cultures of gram-positive cocci not ordinarily associated with burn wound infection. Only in two patients were mixed cultures including aeruginosa seen. It would appear that the entire picture of sepsis in burn patients has become less vivid, with fewer patients involved and fewer mixed infections occurring.

BIOPSIES OF BURN WOUNDS

Biopsies of burn wounds are used in varying degree at different burn treatment centers. The number of biopsies collected in the Institute of Surgical Research in 1980-1981 was at a lower level than has been seen in recent years. Forty-seven patients had wound biopsies collected. The cultural results and incidence of species are shown in Table 8. Pseudomonas aeruginosa, Prov. stuartii, and S. aureus are the bacterial species most frequently encountered, but only P. aeruginosa and Prov. stuartii were recovered with a frequency that suggests a potential epidemic transmission pattern. The decrease in recovery of aureus from the incidence seen in previous years was especially noticeable. Further, no species of the Enterobacteriaceae group was recovered, a marked change from patterns seen several years previous.

CATHETER TIPS AND BACTERIAL CONTAMINATION

Infection following the track of indwelling intravenous catheters has long been a hazard attendant on use of this essential therapeutic

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Table 6. Blood Culture Isolates from 176 Burned Patients: 1 October 1980 - 30 September 1981

Organism	Total No. Isolates	No. Patients Positive
Staph. aureus	11	9
Staph. epidermidis	18	17
Strep. viridans	3	2
Non-hemol. non-Gp D Strep.	2	2
Gp D Enterococcus	2	2
Enterobacter aerogenes	1	1
Klebsiella pneumoniae	10	6
Proteus mirabilis	1	1
Providencia stuartii	31	11
Escherichia coli	4	4
Aeromonas hydrophila	3	1
Citrobacter diversus	1	1
Haemophilus aphrophilus	1	1
Pseudomonas aeruginosa	43	19
Pseudomonas putida	2	2
Corynebacterium sp.	1	1
Candida albicans	1	1
Yeast-like organism	1	1
Fusarium sp.	1	1

Table 7. Blood Culture Isolates in Patients with Mixed Infections
1 October 1980 - 30 September 1981

Organisms	No. of Patients
Coagulase-negative Staph. epidermidis, Non-hemol. Strep. not Gp D	1
Strep. viridans, Non-hemol. Strep. not Gp. D	1
Pseudomonas aeruginosa, Providencia stuartii	1
Pseudomonas aeruginosa, Coagulase-negative Staph. epidermidis	1

Table 8. Bacterial Flora of Biopsies of Burn Wounds of 47 Patients: 1 October 1980 - 30 September 1981

Organism	No. of Patients Positive	No. of Patients with Positive Cultures Who Expired
Staph. aureus	5	3
Streptococcus Gp A	1	1
Streptococcus Gp D		
not Enterococcus	1	1
Streptococcus Gp D		
Enterococcus	3	2
Enterobacter cloacae	1	1
Enterobacter agglomerans	1	1
Klebsiella pneumoniae	3	2
Klebsiella oxytoca	1	1
Proteus mirabilis	1	1
Morganella morganii	1	1
Providencia stuartii	12	4
Escherichia coli	2	0
Citrobacter freundii	1	1
Citrobacter amalonaticus	1	1
Acinetobacter anitratus	1	1
Acinetobacter lwoffii	2	1
Pseudomonas aeruginosa	22	9
Pseudomonas cepacia	1	0
Pseudomonas alcaligenes	1	1
Alcaligenes faecalis	1	0
Candida rugosa	1	1
Candida tropicalis	1	1
Trichosporon beigelii	1	0
Aspergillus sp.	5	3
Alternaria sp.	2	1
Fusarium sp.	2	1
Microsporum sp.	1	1

device in burn patients. Thrombophlebitis following an infected cutdown has been largely controlled by strict limitations on the duration of residence of such catheters. The bacteria recovered from catheter tips cultured on removal from 52 patients are summarized in Table 9. The predominant species numerically were Prov. stuartii, P. aeruginosa and Kleb. pneumoniae. The recovery of 11 different bacterial species from this number of patients suggests that to a considerable extent the contaminants reflect the burn wound flora. However, the conspicuous number of Prov. stuartii strains recovered is consistent with the conclusion that this erstwhile epidemic species is once more appearing in the Institute of Surgical Research burn population as an epidemic opportunistic invader.

Table 9. Bacterial Flora of IV Catheter Tips
1 October 1980 - 30 September 1981

Organism	No. of Isolates	No. of Patients Positive
Staph. aureus	5	4
Staph. epidermidis	5	4
Strep. viridans	1	1
Enterobacter cloacae	2	2
Klebsiella pneumoniae	9	5
Proteus mirabilis	1	1
Morganella morganii	1	1
Providencia stuartii	18	9
Escherichia coli	2	2
Citrobacter freundii	1	1
Pseudomonas aeruginosa	10	9
Candida albicans	4	3
Candida rugosa	2	2
Yeast-like organism	1	1
No. of patients cultured: 52		
No. of cultures: 154		

URINARY TRACT BACTERIOLOGY

Urinary tract infections occur frequently in the severely burned as a consequence of the necessity for indwelling urinary catheters. The results of urine cultures on 142 patients in the reporting period are shown in Table 10. Enteric species were, of course, conspicuous in this series. Klebsiella pneumoniae, Proteus mirabilis, Prov. stuartii, and E. coli were the most frequently encountered Enterobacteriaceae, while P. aeruginosa was a conspicuous offender, while among yeast species Candida albicans was by far the most common species. S. aureus, in contrast to previous years, was relatively uncommon in occurrence.

bacterial species and two of yeasts were recovered. In no instance was more than one species recovered from a sample. Table 11 summarizes the results. Cocci were rare. Enteric species were broadly represented, but only E. coli was recovered from as many as six out of 48 samples. The interesting phenomenon was the relatively large number of species of the genus Pseudomonas recovered. Six species were recorded, with P. putida by far the most frequently encountered species. This number would be even higher if the laboratory had continued speciating all Pseudomonas isolates. However, the expense and time required to continue this differentiation was deemed not justified, since a large amount of data had shown this consistent result. Most of the Pseudomonas sp. listed would fall into the P. putida category. It is noteworthy that P. putida was not recovered even once from the burn wound of a patient. The recovery of this and other uncommon Pseudomonas species from pig skin suggests that this flora is an indigenous part of the pig skin. It does not appear that this xenograft acts as a source of infection in burns.

The review of bacterial flora of burn patients presented here has disclosed certain trends that appear to reflect a marked change in the bacterial flora of the burn patients in this Institute. There was a notable decrease in S. aureus colonization in several sites, with a continued increase in recovery of S. epidermidis, which now occupies a position of numerical frequency that merits further scrutiny as to its potential pathogenicity. Considering the enteric species, there has been a further decrease in invasive infection due to Klebsiella and Enterobacter species, which but a few years earlier were associated with repeated episodes of epidemic sepsis in the burn ward. The reappearance of Prov. stuartii was well documented during the year. This species, after a long period of epidemic colonization of the burn population, disappeared abruptly, and for three years no strains were recovered. In 1979-1980, it reappeared and was far more numerous in 1980-1981. However, the strain(s) now present do not appear to have the virulence or invasiveness that was exhibited by the earlier epidemic strain(s). It is an active colonizer but as yet has only rarely incited sepsis in a severely burned patient.

The consistent performer in the spectrum of species recovered from burn is, of course, P. aeruginosa. In this report, it remains the principal epidemic strain, and its ability to produce sepsis in a severely burned patient remains a conspicuous attribute.

PRESENTATIONS

Lindberg RB: Epidemics of Enterobacteriaceae in burn patient infections. Presented at South African Burn Association Annual Meeting, Skukuza, South Africa, 5 August 1981.

PUBLICATIONS

None.

Table 10. Urine Cultures on 142 Patients
1 October 1980 - 30 September 1981

Organism	No. of Isolates	No. of Patients Positive
Staph. aureus	7	6
Staph. epidermidis	14	11
Strep. viridans	4	4
Non-hemol. Strep. not Gp D	11	11
Gp D Strep. not an Enterococcus	3	2
Gp D Enterococcus	12	10
Enterobacter aerogenes	1	1
Enterobacter cloacae	2	2
Enterobacter agglomerans	2	1
Klebsiella pneumoniae	29	18
Klebsiella oxytoca	1	1
Proteus mirabilis	13	10
Providencia stuartii	33	14
Escherichia coli	69	22
Citrobacter diversus	2	1
Citrobacter freundii	4	1
Acinetobacter anitratus	1	1
Pseudomonas aeruginosa	47	19
Pseudomonas maltophilia	1	1
Pseudomonas cepacia	1	1
Candida albicans	41	12
Candida rugosa	7	6
Candida tropicalis	12	6
Candida krusei	16	1
Trichosporon beigeli	2	1
Torulopsis glabrata	2	1

XENOGRAFT (PORCINE SKIN) CULTURES

Xenografts, in the form of sheets of pig skin from freshly killed hogs, continue to be valuable biologic dressings for management of patients with severe burns. The initial bacteriologic studies of porcine skin showed a relatively low level of contamination in several series. Positive culture rates were below 5%. However, in the past two years, positive culture results have increased markedly. There are no data to indicate that such contaminants give rise to an increased incidence of wound infection, but the bacterial flora of any substance coming in contact with a burn wound should be known in detail. Samples from lots of xenograft assigned to 48 patients in 1980-1981 were cultured. Twenty-two

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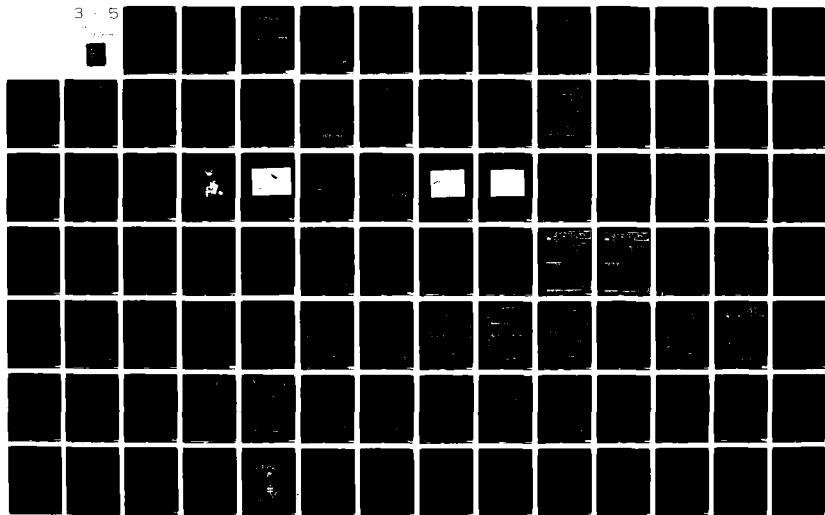


Table 11. Xenograft (Porcine) Cultures from 48 Patients
1 October 1980 - 30 September 1981

Organism	No. of Isolates	No. of Samples Positive
Staph. aureus	3	3
Non-hemol. Strep. not Gp D	1	1
Enterobacter cloacae	1	1
Enterobacter agglomerans	3	3
Klebsiella pneumoniae	5	4
Klebsiella oxytoca	3	2
Klebsiella ozaenae	7	5
Providencia stuartii	5	4
Escherichia coli	12	6
Aeromonas hydrophila	1	1
Citrobacter freundii	2	2
Acinetobacter anitratus	8	3
Acinetobacter lwoffii	5	2
Pseudomonas aeruginosa	20	9
Pseudomonas fluorescens	42	13
Pseudomonas putida	111	38
Pseudomonas maltophilia	2	2
Pseudomonas cepacia	3	2
Pseudomonas stutzeri	6	4
Pseudomonas sp.	88	14
Alcaligenes faecalis	1	1
Corynebacterium sp.	2	1
Candida albicans	1	1
Candida zeylanoides	1	1

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

**REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF
TROOPS WITH THERMAL INJURY -- THE ROLE OF FUNGI IN BURN
WOUND INFECTION: OBSERVATIONS ON BIOPSY AND AUTOPSY
TISSUES FROM SERIOUSLY BURNED SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**Robert B. Lindberg, Ph.D.
Jack R. Henderson, Ph.D.
Susan J. Constable, SSG
Gloria Bailey, SP5**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- THE ROLE OF FUNGI IN BURN WOUND INFECTION: OBSERVATIONS ON BIOPSY AND AUTOPSY TISSUES FROM SERIOUSLY BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1980 - 30 September 1981

Investigators: Robert B. Lindberg, Ph.D.
Jack R. Henderson, Ph.D.
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Biopsy and autopsy samples from burn patients were cultured on Sabouraud's agar. Fungi when isolated were classified to the level of genus. The recovery of fungi was far lower than has been seen in recent years. Genera recovered from both sources included Aspergillus, Alternaria and Fusarium. Pathogenic genera included one strain each of Mucor and of Rhizopus; these were not, however, recovered from patients who developed mucormycosis.

Fungi
Phycomycosis
Burns
Infection
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS
WITH THERMAL INJURY -- THE ROLE OF FUNGI IN BURN WOUND
INFECTION: OBSERVATIONS ON BIOPSY AND AUTOPSY TISSUES
FROM SERIOUSLY BURNED SOLDIERS

Infection of burn wounds by fungi has been long observed. Systemic invasion by a fungus strain, even with a significant degree of burn wound mycosis, is not common and has been primarily caused by members of the Phycomycetaceae. However, microscopic evidence of burn wound invasion by fungi has been frequently observed in our patients, and to document this phenomenon as clearly as possible, routine fungal cultures of all biopsy samples and autopsy tissue samples are performed. Most fungi found in burns are the same species found in the burn ward environment, and burn wound colonization is undoubtedly fortuitous.

FUNGI IN BIOPSIES

During 1980-1981, biopsies from 47 patients were cultured for fungi. The technic developed in this laboratory was used; it consists essentially of placing a small portion of biopsy (at least 100 mg if possible) on Sabouraud's agar in a screw-capped tissue culture bottle. The tissue should touch both agar and bottle wall. Adequacy of sampling has a profound effect on the likelihood of successful recovery of fungi. In general, the tissue should represent the burn-nonburn interface for optimal results. The results in 1980-1981 are summarized in Table 1. The predominant genus in this set was Aspergillus, which has held this spot for at least two years. The numbers recovered can scarcely be regarded as significant. Comparison of recovery rates in recent years with that of 1980-1981 reveals the irregular frequency of positive culture which has occurred; this comparison is shown in Table 2. The number of genera had only once before, in 1975, been as low as four, and in that year only six strains were recovered. In 1980-1981, there were 19 strains recovered; none of the fungal genera recovered was primarily a human pathogen.

FUNGI RECOVERED AT AUTOPSY

Autopsy tissues, including lung, liver, spleen and burn wound samples, are cultured in a manner similar to that used for biopsies. Table 3 summarizes the autopsy collection data. The number of strains recovered was trivial compared to earlier experience. In 1976-1977, for example, 105 strains, comprising 12 genera, were recovered from autopsies. The 23 strains, representing seven genera, would suggest that fungal involvement in the burn wound, at least as it is represented by recovery in culture, is currently of minor concern. The genera recovered can be readily cultured from air in the laboratory, burn ward or autopsy building. Three of the four genera found in biopsies were also found in autopsy samples. Four genera, including the two phycomycetes Rhizopus and Mucor, were found only in postmortem wounds. As with evidence of bacterial invasive infection in 1980-1981, the activity of fungi in the

Table 1. Fungi Recovered from Biopsy Samples - 1980-1981

Genus	No. Patients Positive	No. of Strains Recovered
Microsporium	1	1
Aspergillus	5	10
Alternaria	2	4
Fusarium	2	4
No. of patients cultured: 47		

Table 2. Fungi Recovered from Burn Wound Biopsies - 1973-1981

Genus	Year and No. of Strains Recovered									
	1973	1974	1975	1976-7	1977-8	1978-9	1979-80	1980-1		
<i>Aspergillus</i>	17	5	2	5	23	28	21	10		
<i>Cephalosporium</i>	5	5	1	4	5	5	0	0		
<i>Fusarium</i>	23	17	2	4	4	1	4	4		
<i>Sepodoni</i>	1	0	0	3	0	0	0	0		
<i>Penicillium</i>	1	3	0	1	0	1	2	0		
<i>Alternaria</i>	2	3	1	3	6	1	7	4		
<i>Trichophyton</i>	0	0	0	1	0	0	0	0		
<i>Microsporium</i>	0	0	0	0	0	0	0	1		
<i>Mucor</i>	2	0	0	1	4	0	3	0		
<i>Rhizopus</i>	2	0	0	0	0	0	0	0		
<i>Curvularia</i>	2	3	0	0	0	2	3	0		
<i>Helminthosporium</i>	9	2	0	0	0	1	0	0		
<i>Geotrichum</i>	0	4	0	0	10	0	0	0		
<i>Coccidioides</i>	0	0	0	0	0	1	0	0		
<i>Diplosporium</i>	0	0	0	0	0	0	1	0		
<i>Mycelia sterilia</i>	0	0	0	0	0	2	2	0		
No. patients cultured	106	135	63	113	61	78	75	47		
No. genera	10	8	4	8	6	9	8	4		
No. strains recovered	64	42	6	22	52	42	43	19		

Table 3. Fungi Recovered from Burn Wounds and Viscera at Autopsy, 1980-1981

Genus	Burn Wound No. of Strains	Viscera No. of Strains
Alternaria	4	1
Mucor	1	0
Rhizopus	1	0
Aspergillus	10	0
Penicillium	1	1
Fusarium	2	0
Cephalosporium	2	0

wound appears to have been minimal. However, just as we have no explanation for the decline of mycotic infection, we have no assurance that it will continue to be subdued. Continued observation of these sources is called for.

PUBLICATIONS/PRESENTATIONS - None

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

**REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- SENSITIVITY TO
SULFAMYLON OF PSEUDOMONAS AERUGINOSA RECOVERED FROM
BURNED SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**Robert B. Lindberg, Ph.D.
Virginia C. English, M.A.**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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In 1980-1981, sensitivity to Sulfamylon was assessed on 468 strains of Pseudomonas aeruginosa. There were more strains displaying heightened tolerance than was the case in 1979-1980. Eighty-one percent of the strains tested were inhibited by 0.312% or less of Sulfamylon. The median sensitivity to Sulfamylon was 0.253%, which is higher than that seen in most of the past 10 years, but still the third highest median level seen during that time.

Burns
Pseudomonas
Topical therapy
Sulfamylon
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS
WITH THERMAL INJURY -- SENSITIVITY TO SULFAMYLON OF PSEUDOMONAS
AERUGINOSA RECOVERED FROM BURNED SOLDIERS

Pseudomonas aeruginosa continued, in 1980-1981, to be the principal cause of sepsis in major burns. Although primary burn wound sepsis due to this organism has become rare due to availability of effective topical treatment, invasive systemic infection remains a frequent complication in the later stages of treatment of severe burns. Sulfamylon is relied on as a potent agent capable of controlling Pseudomonas burn wound sepsis, and thus the continued antibacterial effectiveness of this agent is a matter of primary concern. Since the first observations on this drug in this laboratory in 1963, the sensitivity of strains has remained consistent, within a range of deviation that represents a plausible biological fluctuation. Exposure of bacteria to Sulfamylon occurs regularly on the Institute of Surgical Research burn wards, since the drug is used routinely on many patients in the form of a 5% soak, as well as its use in primary treatment as a 10% cream. Exposure of a bacterial species appearing on the ward in a succession of introduced strains, without a marked increase in in vitro resistance over a period of more than 15 years, would suggest that the species is truly immune to such selective change. However, monitoring of sensitivity of patients' strains has been continued. We cannot expect a marked jump in resistance to announce itself; a shift to greater incidence of relatively tolerant strains could be of consequence in clinical use, yet could also be inapparent unless sought out.

SENSITIVITY OF PSEUDOMONAS AERUGINOSA TO SULFAMYLON

Four hundred and sixty-eight strains of P. aeruginosa were tested for sensitivity to Sulfamylon from 1 October 1980 through 30 September 1981. The testing technic has been described in detail in earlier annual reports. It involves an agar dilution technic, with plates poured in concentrations from 5% to 0.019%. Seeded with 1000-cell inocula of broth cultures of recent isolates, the growth-no growth end point is determined at 24 hours. Sensitivity is expressed as the Sulfamylon concentration which inhibits growth.

Sensitivity of the strains tested is summarized in Table 1. The major part of the collection, 81%, of the strains tested were inhibited by 0.312% Sulfamylon or less. This concentration, which is 1/32 of the concentration of Sulfamylon in burn cream, is regarded as well within the limits of sensitivity of microorganisms to Sulfamylon.

It is of especial interest that the continuity of response to Sulfamylon be assessed over a period of several years. Thus short-term fluctuations can be viewed in the framework of the longer view. Sensitivity of Pseudomonas to Sulfamylon over the past 10 years is summarized in Table 2. The recent peak numbers of strains inhibited by the higher concentrations may be observed here. In 1980-1981, the largest number of strains inhibited by an individual concentration was

Table 1. Sensitivity to Sulfamylon of *Pseudomonas aeruginosa*
1 October 1980 - 30 September 1981

No. of Strains	Concentration Required for Inhibition (gm/dl)	% of Total Tested
0	1.250	0
88	0.625	18.8
275	0.312	58.8
46	0.156	9.8
14	0.078	3.0
19	0.039	4.1
9	0.019	1.9
17	< 0.019	3.6
Total 468		

the 275 strains inhibited at 0.312%. This same level inhibited the largest number of strains in 1979-1980. However, in 1978-1979, the largest number was inhibited at the next higher dilution, 0.625%. In 1972, this same phenomenon occurred. It was evident in 1980-1981 that no unusual increase in resistance had occurred, although the median level for sensitivity remained higher than it had been for most of the decade. There were no reports of treatment failure that appeared to be related to the presence of resistant strains.

Variations in sensitivity are most clearly visualized when sensitivity values are arranged on an annual cumulative basis. This information is presented in Table 3. Values are shown for each year since 1968. Annual fluctuations have occurred, but long-term trends can be seen. Through 1970, only a few strains each year needed 0.312% to be inhibited. In 1971, the proportion requiring 0.312% inhibition rose to 82.9%. Starting in 1972, a small number of strains required more than 0.625% for inhibition. However, this number has not progressively increased. It has ranged from 6.5% of all strains tested, in 1979-1980, to 1.0% in 1974. This upper limit tolerance became slightly smaller in 1980-1981. In the range where the vast majority of strains are inhibited, values have fluctuated but have not significantly increased. The picture suggests a slow upward creep in the level of resistance, but the therapeutic effectiveness of Sulfamylon remains high. Relative tolerance of strains from epidemic outbreaks of sepsis suggests that the drug has not changed significantly in its effectiveness.

A more concise but still useful computation of sensitivity in successive annual samplings is to compare median levels of inhibitory activity. This is that concentration below which 50% of strains are inhibited. The values for each year since 1968 are shown in Table 4. Large numbers of relatively tolerant strains will raise this percentage

Table 2. Inhibiting Concentrations of Sulfamylon for *Pseudomonas aeruginosa*, 1971-1981

Year	No. of Strains	Concentration of Sulfamylon in gm/dl; No & % of Strains Inhibited							
		1.25	0.625	0.312	0.156	0.078	0.039	0.019	< 0.019
1971	280	0	48	41	56	57	65	13	0
			17.1	14.6	20.0	20.4	23.2	4.6	
1972	463	29	212	46	88	31	37	15	5
		6.3	45.8	9.9	19.0	6.7	8.0	3.2	1.1
1973	285	4	14	85	85	52	32	12	1
		1.4	4.9	29.8	29.8	18.3	11.2	4.2	0.4
1974	437	5	59	78	97	97	86	11	4
		1.1	13.5	17.8	22.2	22.2	19.7	2.5	0.9
1975	656	13	133	108	155	68	147	28	4
		2.0	20.3	16.5	23.6	10.4	22.4	4.3	0.6
1976-77	698	4	118	135	295	95	18	23	10
		0.6	16.9	19.3	42.3	13.6	2.6	3.3	1.4
1977-78	141	16	17	16	26	48	12	5	1
		11.3	12.1	11.3	18.4	34.0	8.5	3.5	0.7
1978-79	715	78	307	193	59	47	16	1	14
		10.9	42.9	27.0	8.3	6.6	2.2	0.1	2.0
1979-80	461	30	98	178	68	45	25	13	4
		6.5	21.3	38.6	14.8	9.8	5.4	2.8	0.9
1980-81	468	0	88	275	46	14	19	9	17
			18.8	58.8	9.8	3.0	4.1	1.9	3.6
TOTAL	4604	179	1094	1155	975	554	457	130	60
		3.9	23.8	25.1	21.2	12.0	9.9	2.8	1.3

Table 3. Cumulative Sensitivity to Sulfamylon of Pseudomonas aeruginosa, 1968-1981

Year	No. of Strains	Concentration of Sulfamylon in gm/dl; % of Strains Inhibited						
		1.25	0.625	0.312	0.156	0.078	0.039	0.019 < 0.019
1968	294	100	100	95.1	60.4	45.8	14.1	1.7 0
1969	385	100	100	96.5	50.0	26.9	7.7	0.5 0
1970	296	100	100	100	78.0	49.9	21.9	2.0 0
1971	280	100	100	82.9	68.3	48.3	27.9	4.7 0
1972	463	100	93.7	48.0	38.0	19.0	12.3	4.3 1.1
1973	285	100	98.1	81.3	57.0	33.5	16.1	3.2 0.4
1974	437	100	99.0	85.5	67.5	45.3	23.1	2.4 0.9
1975	656	99.8	97.8	80.1	63.2	38.9	24.2	5.0 0.6
1976-77	698	100	99.4	82.5	63.2	21.0	7.3	4.7 1.4
1977-78	141	100	98.1	83.5	64.3	34.3	17.9	4.5 0.9
1978-79	715	100	95.8	71.5	52.2	28.7	15.2	3.8 1.1
1979-80	461	100	93.5	72.2	33.6	18.8	9.0	4.4 0.8
1980-81	468	100	95.9	81.2	22.4	12.5	9.5	5.5 3.6

Table 4. Median Value of Pseudomonas aeruginosa
Sensitivity to Sulfamylon, 1968-1981

Year	No: of Strains Tested	Median Inhibitory Level (gm/dl)
1968	294	0.136
1969	385	0.176
1970	296	0.068
1971	280	0.125
1972	463	0.316
1973	285	0.111
1974	437	0.086
1975	656	0.125
1976-77	698	0.117
1977-78	141	0.089
1978-79	715	0.324
1979-80	461	0.198
1980-81	468	0.253

value; fewer such strains in an assessment period will lower it. It will be seen that the three highest years in order of inhibiting level were 1972 (0.316%), 1978-79 (0.324%) and 1980-81 (0.253%). In no other year did the mean inhibiting value reach 0.2%. In general, the median value fluctuated without any long-term trend toward increase. Particular note should be taken of the past three years, however. The values went from 0.324% in 1978-79 to 0.198% in 1979-80, to 0.253% in 1980-81. This suggests that particular attention be paid to the sensitivity of current isolates and to the response to therapy of Sulfamylon-treated patients in the immediate future. There is no objective cause for alarm, but the clustering of three years of higher median values has not occurred before, in all the time such measurements have been made. We must remain aware of the lability of bacterial species and of the possibility that an increase in resistance to Sulfamylon in strains of P. aeruginosa could be occurring. In view of the history of the use of Sulfamylon in burns, this does not appear to be especially likely, but the possibility should not be ignored. While routine sensitivity testing of Pseudomonas isolates has not been carried out, it is entirely feasible, and it would offer an assurance that treatment of a relatively resistant strain was not being undertaken unknowingly.

PRESENTATIONS/PUBLICATIONS - None

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

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OTHER GRAM-NEGATIVE BACILLI IN BURNED SOLDIERS: NEW
POTENTIAL OPPORTUNISTIC PATHOGENS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1980 - 30 September 1981

Investigators:

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

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Cultures from clinical specimens are scrutinized with specific attention paid to unusual oxidative and fermentative gram-negative bacilli. Several such forms have shown capacity to incite institutional epidemics of sepsis among burn patients. Examples would include Providencia stuartii, Enterobacter cloacae, and Klebsiella oxytoca. New species, not previously observed as part of the burn flora, are thus noted and added to the extensive roster of opportunists which can colonize burn wounds. In the 1980-1981 period, 21 species were regarded as meriting the epithet "unusual." No new epidemic strains were noted.

Burns
Oxidative microorganisms
Pseudomonas
Acinetobacter

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH
THERMAL INJURY: NON-FERMENTATIVE AND OTHER GRAM-NEGATIVE BACILLI
IN BURNED SOLDIERS: NEW POTENTIAL OPPORTUNISTIC PATHOGENS

Observations over an extended period of the burn wound flora indicate that the species distribution is essentially opportunistic. There are strain differences in such groups as the Enterobacteriaceae which can lead to establishment of epidemic episodes in which invasive infection may be caused by a species previously seen only as an innocuous colonizer. There appear to be no intrinsic barriers to any given species to pass from an opportunistic colonizer to an invasive pathogen, but the number of species that have done this is fortunately not great. The incidence of oxidative organisms and of rarely encountered enteric species has been followed carefully for several years. Strains from various clinical sources are compiled.

The unusual gram-negative species recovered in 1980-1981 are summarized in Table 1. Some species previously recorded as uncommon (i.e. Providencia stuartii) were not included this year because they were not any longer uncommon. Twenty-one species were recognized, only one of which occurred in large numbers. This was Pseudomonas putida, which was recovered in large numbers from frozen porcine xenograft sheets. There were six species of Pseudomonas recovered. Only one, P. putida, was recovered from blood samples. The patient did not develop sepsis. The remaining species were found rarely on wound and in sputum. Their most common site, however, was from porcine xenograft. These relatively uncommon species of Pseudomonas were most probably brought into the burn wards on such xenograft. Aeromonas hydrophila, which has been an infrequently recovered species in previous years, was three times recovered from blood culture. Acinetobacter anitratus and Acineto. lwoffii were recovered in equal numbers, primarily from sputum and wound cultures. Neither was present in conspicuous numbers. Increase in these species, when it has occurred, has always been traceable to an admission or set of admissions from a different geographic area. Citrobacter sp. were observed with especial care since they have been shown to be capable of inciting episodes of epidemic sepsis in burn wards. In this series, only Citrobacter diversus was numerically prominent. No septic episodes occurred, but this species has been the cause of brief epidemic sepsis periods.

Prior to 1970, unusual oxidative gram-negative bacteria were frequently recovered in tissues of fatally burned patients at autopsy. These isolates were less common in recent years. In 1980-1981, only two species of the total observed were found in autopsy samples. This discrepancy is so wide that it suggests a technical change or some alteration in the procedure of collecting and processing specimens rather than total absence of these species in tissues at autopsy. No precise change can be pointed out at this time, but this topic should be investigated to resolve the marked discrepancy now being documented.

Table 1. Unusual Gram-Negative Species Recovered from Clinical Bacteriology Specimens
1 October 1980 - 30 September 1981

Organism	Source and Number of Isolates							Total
	Wound	Blood	Respiratory	Urine	Biopsy	Xenograft	Autopsy	
<i>Pseudomonas fluorescens</i>	3	0	1	0	0	42	0	46
<i>Pseudomonas putida</i>	5	2	3	0	0	111	0	121
<i>Pseudomonas maltophilia</i>	0	0	0	1	0	2	0	3
<i>Pseudomonas cepacia</i>	2	0	1	1	0	2	0	6
<i>Pseudomonas stutzeri</i>	0	0	0	0	0	6	0	6
<i>Pseudomonas alcaligenes</i>	1	0	0	0	0	0	0	1
<i>Alcaligenes faecalis</i>	2	0	0	0	1	0	0	3
<i>Aeromonas hydrophila</i>	4	3	1	0	0	1	1	10
<i>Achromobacter xylosoxidans</i>	0	0	1	0	0	0	0	1
<i>Flavobacterium</i> sp.	1	0	0	0	0	0	0	1
Group 2K-1	1	0	0	0	0	0	0	1
<i>Haemophilus aphrophilus</i>	0	1	0	0	0	0	0	1
<i>Acinetobacter lwoffii</i>	4	0	9	0	2	5	0	20
<i>Acinetobacter anitratus</i>	3	0	7	1	1	8	0	20
<i>Citrobacter diversus</i>	5	1	10	2	0	0	0	18
<i>Citrobacter freundii</i>	2	0	1	4	0	2	0	9
<i>Citrobacter amalonaticus</i>	0	0	0	0	1	0	0	1
<i>Hafnia alvei</i>	0	0	3	0	0	0	0	3
<i>Klebsiella ozaenae</i>	2	0	2	0	0	7	0	11
<i>Klebsiella oxytoca</i>	10	0	27	1	1	3	22	64
<i>Enterobacter agglomerans</i>	0	0	7	2	1	3	0	13

No incipient epidemic-causing species is to be incriminated on the basis of this compilation. However, the potential for new opportunistic invaders to arise from this heterogeneous collection of opportunistic colonizers remains a possibility. These forms should be continually monitored.

PRESENTATIONS/PUBLICATIONS - None

ANNUAL PROGRESS REPORT

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UNCLASSIFIED

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An ongoing basic study of enzyme production by Pseudomonas aeruginosa has been carried on to determine, if possible, distinctive enzyme production patterns that might be associated with virulence of this species. No other attribute thus far demonstrated has been shown to correlate with virulence as it is demonstrated by ability to incite invasive sepsis of a burn wound. In 1980-1981, enzymes previously assessed and continued included caseinase, amylase, elastase, lipase and lecithinase. Systems were added to assess fibrinolysin, hyaluronidase and chondroitin sulphatase. Collagenase was sought by several means, but could not be demonstrated. Correlations of enzyme patterns and virulence will be the object of continued search.

Burns
Pseudomonas
Virulence
Enzymes
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH
THERMAL INJURY -- ENZYME PRODUCTION AND VIRULENCE OF PSEUDOMONAS
AERUGINOSA RECOVERED FROM SOLDIERS WITH THERMAL INJURY

Despite the use of effective topical chemotherapeutic agents, threat of invasive sepsis due to Pseudomonas aeruginosa remains a significant hazard to the burn patient. Mechanisms of bacterial invasion of burn patients have not been elucidated to a significant degree, and study of possible enzyme-related mechanisms has been pursued. Reports have suggested that bacterial exoenzyme production, particularly proteases, may be a possible cause of the pathogenesis of P. aeruginosa (1-3). Further, several potent reversible and irreversible enzyme inhibitors have been described. It has been postulated that such inhibitors may be of value in the future for the clinical treatment of P. aeruginosa infections (4).

During 1980-1981, 468 isolates of Pseudomonas from burn patients were examined in this laboratory for their ability to produce caseinase, amylase, elastase, lipase and lecithinase. Previous reports have summarized the media and substrates used in plate assays for detection of Pseudomonas enzyme production (5-6).

In this study period, assays were added for detection of fibrinolysin, hyaluronidase and chondroitin sulphatase production by Pseudomonas. Plate assay technics, as described by Janda et al (7), were used for the detection of the production of these enzymes by 72 Pseudomonas isolates from burn patients.

1. Kawaharajo K, Homma JY, Aoyama Y, Morihara K: In vivo studies on protease and elastase from Pseudomonas aeruginosa. Jpn J Exp Med 45: 89-100, 1975.
2. Kawaharajo K, Homma JY, Aoyama Y, Okada K, Morihara K: Effects of protease and elastase from Pseudomonas aeruginosa on skin. Jpn J Exp Med 45:79-88, 1975.
3. Liu PV: Extracellular toxins of Pseudomonas aeruginosa. J Infect Dis 130 (Suppl): S94-S99, 1974.
4. Nishino N, Powers JC: Pseudomonas aeruginosa elastase. Development of a new substrate, inhibitors and an affinity ligand. J Biol Chem 255:3482-3486, 1980.
5. English VC, Lindberg RB: Enzyme production and virulence of Pseudomonas aeruginosa recovered from soldiers with thermal injury. USAISR Annual Report FY 1979, BAMC, Ft Sam Houston, Texas, pp 179-183.
6. English VC, Lindberg RB: Enzyme production and virulence of Pseudomonas aeruginosa recovered from soldiers with thermal injury. USAISR Annual Report FY 1980, BAMC, Ft Sam Houston, Texas, pp 159-163.
7. Janda JM, Böttone EJ: Pseudomonas aeruginosa enzyme profiling: Predictor of potential invasiveness and use as an epidemiological tool. J Clin Microbiol 24:55-60, 1981.

Fibrinolysin was measured by incorporating 280 mg of human fibrinogen (Sigma) into 100 ml of nutrient agar (Difco). Following spot inoculation of the medium, the plates were incubated for 24 hours at 37° C. Clear zones showing a diameter of greater than 2 mm around bacterial growth indicated lysis of human fibrinogen.

Production of hyaluronidase and chondroitin sulphatase was measured by the appropriate substrate which was incorporated into brain heart infusion agar (Difco). Hyaluronic acid from human umbilical cord (Sigma) was added to the base medium so that the final concentration was 400 µg/ml. Similarly, chondroitin sulphate from whale cartilage (Sigma) was prepared to achieve a substrate concentration of 400 µg/ml in brain heart infusion agar. Both media were supplemented with bovine albumin (Sigma), 1 gm/dl. After inoculation and 48 hours of incubation at 37° C, the plates were flooded with 2N acetic acid. After 10 minutes, the excess acetic acid was removed and positive reactions were determined by the presence of a clear zone around the inoculum.

The results of tests performed on P. aeruginosa isolates are shown in Table 1. Caseinase, lipase and lecithinase were produced by 97.2%, 89.1% and 82.9%, respectively, of 468 isolates tested. There were no isolates which produced amylase. Fibrinolysin was produced by 97.2% of 72 isolates tested. Neither hyaluronidase nor chondroitin sulphatase production could be demonstrated.

It is interesting to note that only 14.1% of P. aeruginosa were able to produce elastase during 1980-1981, compared to 86% of the strains tested in 1979-1980. Table 2 summarizes elastase activity of P. aeruginosa from various sources. Pseudomonads cultured from wound areas (swab cultures, biopsies and contact plates) were the most frequent elastase producers when compared to other body sources from which Pseudomonas was isolated. It was in June 1980 that the recovery of elastase-producing isolates of Pseudomonas began to decline. Although the decline was gradual, few strains which produce elastase are currently being recovered. This shift in the elastolytic characteristic of clinical isolates of Pseudomonas is a probable reflection of changes in the population of the Pseudomonas flora in the burn ward. Such biophysiological changes have been noted among Pseudomonas and other bacterial species in the past. It is likely that the incidence of Pseudomonas capable of producing elastase and other enzymes will continue to fluctuate with changes in the population of the burn ward flora.

A particular pattern of enzyme production could not be established when comparing individual Pseudomonas isolates. Isolates from an individual patient showed a tendency toward like patterns of enzyme production, but dissimilar patterns were found also.

Efforts to demonstrate collagenase activity by P. aeruginosa isolated from burn ward patients were unsuccessful. Collagenolytic activity of anaerobic bacteria is well established. Among aerobic bacteria, true collagenolytic activity has not been conclusively demonstrated. Waldvogel

et al (8) reported one strain of Staphylococcus aureus which was capable of lysing collagen when grown under anaerobic conditions. However, he was not able to demonstrate collagenase production by three strains of P. aeruginosa when grown under strict anaerobic conditions in a liquid medium containing nitrate as a terminal electron acceptor.

Carrick et al (9) reported the recovery of a protease capable of catalyzing the hydrolysis of insoluble collagen from one strain of P. aeruginosa. The culture of Pseudomonas was grown under aerobic conditions in 5% peptone and 0.25% trypticase soy broth. Enzyme recovery was accomplished by use of chemical and chromatographic techniques.

Selected strains of P. aeruginosa isolated from burn ward patients of the Institute of Surgical Research were screened for collagenase production. Testing was carried out under aerobic and anaerobic conditions utilizing both liquid and solid medium with appropriate nitrate concentration. Collagenolytic activity of these strains was not demonstrated. If P. aeruginosa is capable of hydrolyzing native collagen, the true test for collagenolysis, it appears to be an attribute of relatively few strains.

Enzyme profiles of P. aeruginosa isolates from burn ward patients will be expanded to include tests for production of gelatinase, fibrin coagulase and hemolysin.

8. Waldvogel FA, Swartz MN: Collagenolytic activity of bacteria. J Bacteriol 98:662-667, 1969.

9. Carrick L, Berk RS: Purification and partial characterization of a collagenolytic enzyme from Pseudomonas aeruginosa. Biochim Biophys Acta 391:422-434, 1975.

PRESENTATIONS/PUBLICATIONS - None

Table 1. Summary of Enzyme Production by 468 Pseudomonas Isolates
1980-1981

		ENZYME							
		Caseinase	Amylase	Elastase	Lipase	Lecithinase	Fibrinolysin	Hyaluronidase	Chondroitinase
No. of isolates tested	468	468	468	468	468	468	72	72	72
No. of positive tests	455	0	66	417	388	70	0	0	0
% of isolates positive	97.2	0	14.1	89.1	82.9	97.2	0	0	0

Table 2. Distribution of Elastase-Producing *Pseudomonas* Isolates Among Their Sources of Origin - 1980-1981

	SOURCE OF CULTURE				
	Blood	Sputum	Postmortem viscera	Urine	Wound*
No. of isolates tested	27	150	42	48	201
No. of elastase positive isolates	3	15	0	5	43
% positive isolates	11.1	10.0	0	10.4	21.3

* Includes swabs, biopsies, and contact plates.

FINAL REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

**REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF
TROOPS WITH THERMAL INJURY -- EXPERIMENTAL INHALATION
INJURY IN THE GOAT**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**Harrel L. Walker, M.S.
Charles G. McLeod, Jr., D.V.M., LTC, VC
William F. McManus, M.D., COL, MC**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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Inhalation injuries are usually produced by inhalation of gaseous or particulate products of incomplete combustion and are rarely due to heat per se unless steam is inhaled. The clinical and anatomic characteristics of an appropriate animal model should mimic the disease encountered clinically. A model of inhalation injury has been produced in anesthetized goats through the use of a modified bee smoker. The smoke is delivered at a low temperature and contains by-products of incomplete combustion.

This reproducible injury produces necrotic tracheobronchitis and bronchiolitis with pseudomembrane and cast formation in association with mild multifocal atelectasis and bronchopneumonia. These lesions spontaneously resolve within three weeks without supportive therapy. The upper trachea, protected from smoke injury by the inflated cuff of the endotracheal tube, showed no evidence of injury. This nonlethal injury is proposed as an appropriate model for evaluation of the pathophysiology and treatment of inhalation injury.

**Goats
Smoke
Inhalation injury
Burn injury
Nonlethal injury**

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- EXPERIMENTAL INHALATION INJURY IN THE GOAT

The incidence of inhalation injury in patients admitted to burn centers has been estimated to be from 15 to over 30% (1). In a study of 100 patients admitted to the University of Wisconsin Burn Center and the Duke University Burn Service, 33 showed bronchoscopic evidence of inhalation injury (2). In spite of the remarkable recovery capacity of the respiratory system, the mortality rate in such cases is estimated to be from 48 to 86% (3). In patients with extensive burns, the mortality rate of those with inhalation injury exceeds that expected for patients without such injury (4).

Inhalation injuries commonly take the form of an inflammatory tracheo-bronchitis due to inhalation of gaseous or particulate products of incomplete combustion and are rarely due to heat alone unless steam has been inhaled (5). These toxic substances include acetic acid, acetic anhydride, oxides of nitrogen, acrolein, sulphur dioxide, formaldehyde and cyanide.

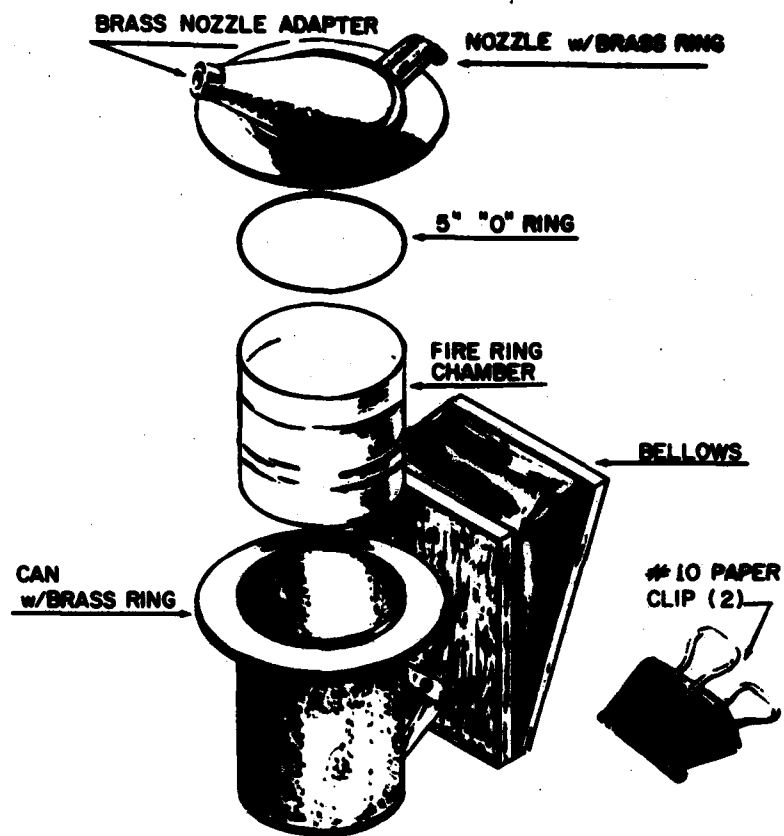
As a result of improved techniques of early resuscitation, burn patients rarely succumb to shock or renal failure, which in the past were major causes of early death. The impact of inhalation injury has become more prominent and emphasizes the need for an animal model of the injury independent of any complications caused by cutaneous burns.

This report describes a model of inhalation injury in the goat in which reproducible nonlethal inhalation injury accompanies histologic changes which occur in a predictable manner. The clinical and anatomic characteristics of this model compare favorably with those of inhalation injury in man.

MATERIALS AND METHODS

Construction and adaptation of bee smoker for producing inhalation injury. Inhalation injury was produced in anesthetized goats using a

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GOAT SMOKER
MODIFIED FROM BEE SMOKER

Figure 1

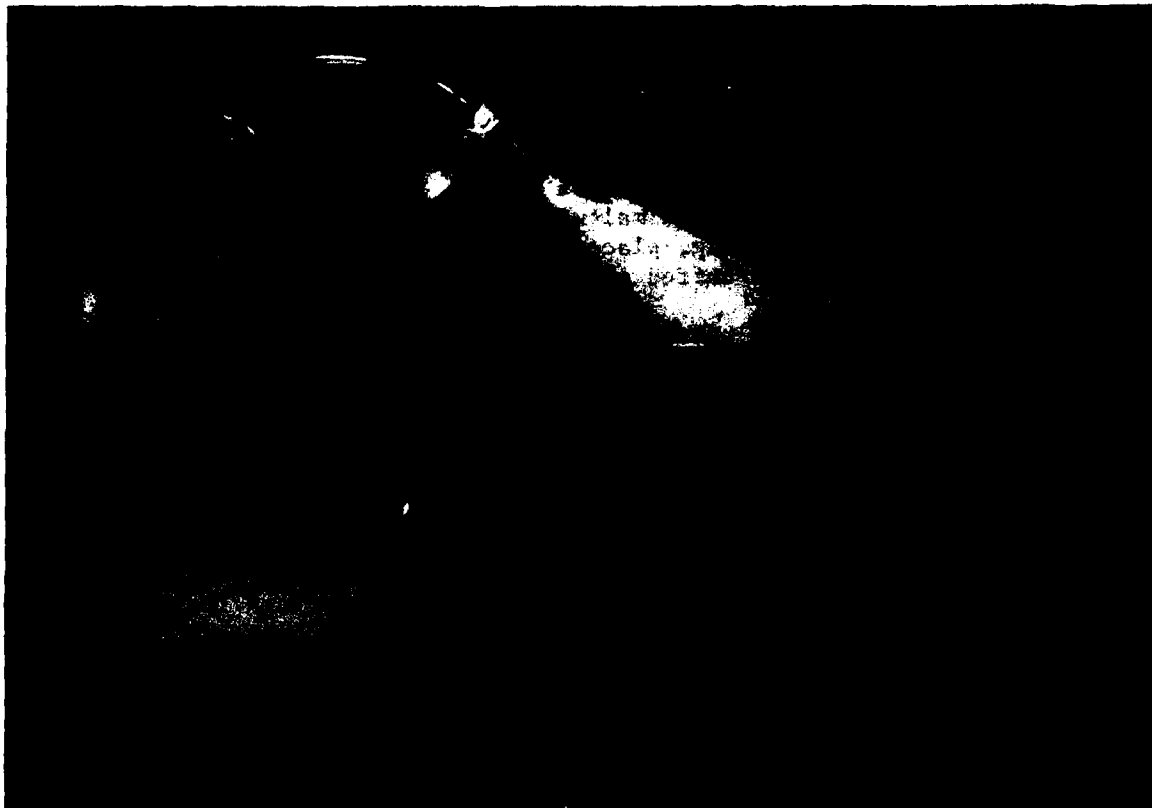


Figure 2. Smoker with endotracheal tube attached.

modified bee smoker. A standard-sized bee smoker (model number N-3) manufactured by A.R. Root Company^R, San Antonio, Texas, was adapted for attachment to an endotracheal tube. The nozzle of the smoker was removed from the can or body of the smoker and a brass adapter soldered to the opening of the nozzle. This brass adapter (3/4" long, 1" OD with a 5/16" ID) served to connect the endotracheal tube to the smoker nozzle. To prevent leakage of smoke, one of two brass rings (OD 5-3/4" and ID 4-1/4") was soldered to the base of the nozzle and the other to the top of the can or body of the bee smoker (Fig. 1). A 5" neoprene (chloroprene rubber) ring was placed between the two brass rings, and the rings were held firmly together and the nozzle secured on the can with two #10 paper clips (Fig. 2).

Method of inhalation injury (nonlethal). Twenty-two goats ranging in weight from 18 to 44 kg were used in this study. Food was withheld 24 hours prior to any study. Each animal was anesthetized with intravenous methohexital sodium, 10-15 mg/kg, placed in ventral recumbency on a flat surface and intubated with a cuffed endotracheal tube of appropriate size.

The fire chamber of the bee smoker was half filled with dyed cotton towel waste, a fuel which resulted in uniform pulmonary injury. After ignition of the fuel, the smoker was connected to the endotracheal tube and smoke delivered to the lungs by pushing the bellows 15 times. This procedure was repeated a total of four times, checking each time for adequate smoke production by the ignited waste, with a time lapse between each repetition of 1-1/2 to 2 minutes.

Method of temperature monitoring. All temperature measurements were taken with a Tele-Thermometer (YSI Model 43SC), Yellow Springs Instrument Co., Inc. The sensor end of the Tele-Thermometer probe was placed in the distal end of the endotracheal tube for measuring temperatures of the animal's trachea, the smoke produced by the smoking device and the ambient air of the working area.

Using the device without fire to measure the temperature of the ambient air at the distal end of the endotracheal tube prior to intubation of the animal, it was found to be 26.5° C. After intubation of the animal, the temperature of the air rose to 37° C at the distal end of the endotracheal tube.

Prior to intubating the animal, the temperature of the smoke produced by the device was measured and found to be 46° C at the distal end of the endotracheal tube. With the animal intubated, the temperature of the smoke delivered to the distal end of the endotracheal tube dropped to 39° C.

Pathology. For gross and microscopic studies of the lungs and tracheobronchial tree the animals were sacrificed on days 1, 2, 3, 5, 6, 7, 8, 9, 10, 14 and 15. Tissue samples were processed routinely for light microscopy.

RESULTS

No animals died from this procedure. They were eating and drinking normally within 24 hours after injury. Smoke exposure regularly produced

necrotic tracheobronchitis and bronchiolitis with pseudomembrane and cast formation (Fig. 3). Sloughing casts composed of necrotic respiratory epithelium (Fig. 4) occurred and were associated with a mild multifocal atelectasis and bronchopneumonia. These lesions resolved within three weeks without supportive therapy. In each goat, the upper segment of the trachea, which was protected from smoke injury by the inflated cuff on the endotracheal tube, showed no evidence of injury. Intubated control animals which were not insufflated with smoke showed no significant lesions.

DISCUSSION

Moritz and his colleagues (5) observed in dogs that only when the temperature of heated air alone was high enough to produce instantaneous burning of the skin and upper respiratory mucosa was there sufficient residual heat in the air reaching the lungs to cause pulmonary injury. The same investigators experimented with inhaled steam; the resultant pulmonary injury was severe, and the animals survived only for a period of 10 hours. Hot air, flame, blast, and steam were used to produce inhalation injury in animals by these investigators, and smoke was never included as part of their studies.

A murine model of inhalation injury has been described by Zawacki et al (6). In this model, burned and unburned mice exhibited reproducible responses and mortality following controlled exposures to smoke. However, burned mice receiving a 4-minute dose of 85° C smoke failed to develop major histological changes in the trachea or lungs even though they lived several days before death occurred.

In earlier prototype studies, a lethal inhalation injury in the goat was produced using an endotracheal tube 70 cm in length. The tip of the endotracheal tube extended to the carina of the trachea in the animal of the size used. The described smoking procedure using such an endotracheal tube resulted in a severe injury causing death of the animal in 12 to 18 hours. The rapid demise of such animals made study difficult and bore little resemblance to the clinically encountered disease. The experimental model of inhalation injury here described reliably and consistently produces histological changes in the tracheobronchial tree resembling those seen in patients. The nonlethal inhalation injury as described will permit useful assessment of the pathophysiology and treatment of such injury. Infliction of both inhalation injury and cutaneous burn injury in the study animal may even allow one to study the interaction between such injuries.

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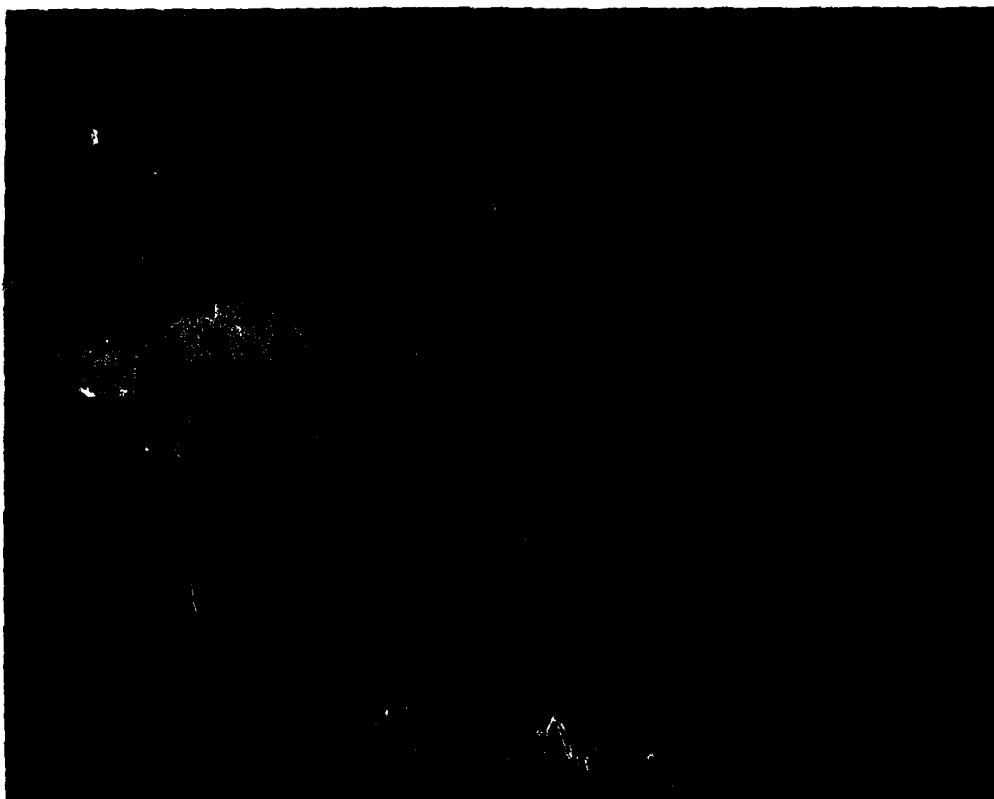


Figure 3. Trachea with sloughing necrotic mucosa. Darkened subpleural foci (arrow) represent atelectasis and mild pneumonia.

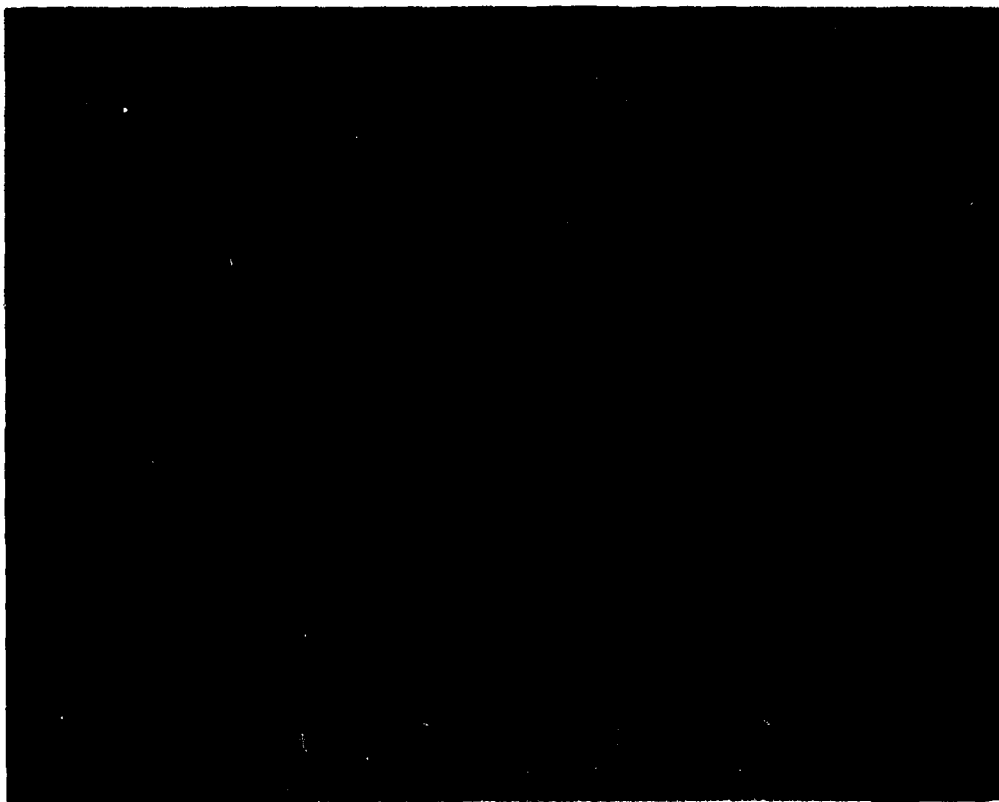


Figure 4. Necrotic cellular casts (arrows) in bronchi of sections cut through right cranial lobe. Darkened areas represent atelectasis and mild acute bronchopneumonia.

FINAL REPORT

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1 October 1980 - 30 September 1981

Investigators:

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UNCLASSIFIED

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In the continuing search for methods to achieve control of Pseudomonas aeruginosa and other burn wound infections, we have examined an experimental sterilant containing sodium chlorite and lactic acid, in a hydroxyethyl cellulose polymer gel. This sterilant exerts significant control of Pseudomonas aeruginosa infection with treatment 1 hour post-burn in a well-characterized animal model. These experiments indicate that greater protection can be achieved in the burn-infected animals when treatment is delayed for 24 hours. Previous experiments with aqueous chlorine compounds by other investigators and these experiments with a hydroxyethyl cellulose polymer gel containing chlorine show that they can control infections. No significant toxicity was demonstrated by sodium chlorite-lactic acid gel in animals with 60% total body surface burns when treated for 10 days with concentrations of sodium chlorite-lactic acid gel up to 0.8%.

Rats
Burn injury
Pseudomonas
Chemotherapy
Sodium chlorite-lactic acid gel
Burn wound sepsis

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL
INJURY -- TOPICAL USE OF SODIUM CHLORITE-LACTIC ACID GEL IN PSEUDOMONAS
BURN WOUND SEPSIS

Topical chemotherapy is widely used for the control of bacterial flora in burn wounds. Infection of burn wounds is controlled to a varying extent by topical use of silver nitrate, silver sulfadiazine or mafenide acetate in most burn centers. These drugs do not entirely preclude burn infection, and such infection continues to be a cause of death in burn patients. With emerging microbial resistance to some agents and undesirable side effects with others, exploration of new antimicrobials is warranted.

Sodium chlorite-lactic acid gel (supplied by Alcide Corporation, 38 East Mall, Plainview, New York 11803) is an experimental sterilant lethal to Staphylococcus, Klebsiella, Enterobacter, Pseudomonas and Escherichia in vitro. In this study, this sodium chlorite-lactic acid combination was used in gel form for the topical treatment of experimental Pseudomonas aeruginosa burn wound sepsis. The gel was used as a two-part drug, part A containing sodium chlorite and part B containing lactic acid. In the mixed gel, the acid releases chlorine dioxide, the principal active agent. (See Annex I for the formulation of sodium chlorite-lactic acid gel.)

Chlorine compounds are not new to wound or burn therapy. In World War I, battlefield wound infections were treated topically with Dakin solution or 0.5% sodium hypochlorite. This approach to wound infection was developed by Alexis Carrel, a surgeon, and Henry D. Dakin, a chemist (1). The wounds Carrel treated were deep, jagged, filled with dead and dying tissue, soaked in wound secretions, and often contained dirt; the idea of applying an antiseptic to such wounds seems natural. The difficult problem, then as now, was to find an antiseptic with sufficient potency to destroy microorganisms which was not harmful to healthy tissue or toxic to the patient. The task of finding such an antiseptic fell to Dakin. Since the introduction of Dakin solution, many changes have been made in its composition and formulation.

The use of Dakin hypochlorite solutions for the topical chemotherapy of burns was described by Bunyan in World War II (2). He employed envelopes of silk impregnated with Bakelite which were placed over wounds on the arms, legs or torso. The envelopes were sealed and irrigations were done through openings; it was not necessary to disturb dressings to treat the wounds. This method was reported to relieve pain, reduce inflammation and control primary infections, but has never achieved any wide acceptance.

-
1. Burdick AS: The Dakin chlorine-carrying antiseptics. Am J Clin Med 25:749-755, 1918.
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Milton solution containing 1% sodium hypochlorite and 16.5% sodium chloride (3) has been used as a topical antimicrobial agent. It was reported to accelerate separation of the eschar, permit earlier skin grafting and reduce bacterial counts in a majority of patients without producing significant side effects. The use of an irrigation technique enabled this solution to be applied regularly without the need for frequent changing of dressings.

Pluronic gel F-127 was reported to be an effective base for the topical application of silver nitrate or silver lactate on burns covering 18% to 22% of the total body surface in rats (4). Some protection was afforded by the Pluronic gel alone. Incorporation of antimicrobial agents enhanced the effectiveness of the dressing.

The successful control of *P. aeruginosa* burn wound sepsis by use of mafenide acetate burn cream was demonstrated both in an animal model (5,6) and in burn patients by Lindberg and his colleagues in 1968 (7). These studies showed diminished mortality in burn patients; most of the improvement occurred in burns involving 30% to 60% of the total body surface. There was no improvement of survival in patients with burns greater than 60% of the total body surface.

In a 1966-1970 study of 350 patients at the Shriners Burn Institute and Cincinnati General Hospital, Cincinnati, Ohio, topical gentamycin and silver sulfadiazine were evaluated in a parallel study and were reported to produce better results than silver nitrate (8). That study also showed that none of the agents exerted a significant influence on survival in patients with burns exceeding 50% of the total body surface area.

Laboratory studies show that mixtures of sodium chlorite and lactic acid have a wide range of bactericidal activity, controlling gram-positive bacteria and gram-negative bacteria. The purpose of the present study was to test the control of *P. aeruginosa* burn wound sepsis by this combination in a well-characterized model in the rat (5,6).

MATERIALS AND METHODS

Adult, Sprague-Dawley (Holtzman strain) rats were used throughout

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this investigation. All animals were conditioned, anesthetized, burned and seeded as previously reported (5,6). This procedure involves a uniform full-thickness, nonlethal burn covering 18% to 22% of the total body surface which results in high mortality when seeded with P. aeruginosa.

A second burn procedure was used to produce a 60% full-thickness injury. This was accomplished by a 10-second exposure of an area of the dorsum to boiling water and exposure of an area of the abdomen for 2.5 seconds to boiling water. Prior to burning the abdomen, 10 ml of normal saline was given by intraperitoneal injection to prevent the burning of the muscle layer and visceral organs and to accomplish fluid resuscitation. This 60% burn alone produces about 20% mortality. After burning, water and food were provided ad libitum for all test animals. An ambient temperature range of 21° C to 27° C was maintained. Survivors were observed for 30 days after seeding or seeding and treatment.

Organisms. P. aeruginosa, strain ISR 12-4-4 (59) was the challenge microorganism employed throughout the study. This microorganism was isolated from the blood culture of a patient who expired with septicemia following second- and third-degree burn trauma over 70% of her body. A sufficient quantity of this microorganism was grown in evaporated milk, frozen and placed in storage at -80° C for preservation. Cultures for use in the entire series of experiments were prepared from the frozen preserved samples; 0.1 ml of the microorganism-milk mixture was placed in 9.9 ml of trypticase soy broth and incubated for 18 hours prior to use.

Challenge techniques. The usual seeding culture contained 10^8 organisms per ml, and 1 ml of this 18-hour culture was used. The standard area topically seeded was 64 cm² for a 20% burn and 96 cm² for all burns greater than 20%. The dose was evenly distributed over the entire burned area.

Single treatment with sodium chlorite-lactic acid gel. This experiment was designed to evaluate the antimicrobial activity of sodium chlorite-lactic acid gel with only one application, and to determine whether a single delayed treatment with sodium chlorite-lactic acid gel had any beneficial effect.

Treatment prior to seeding. Three drugs were evaluated as a treatment prior to seeding with P. aeruginosa. Mafenide acetate, silver sulfadiazine and sodium chlorite-lactic acid gel were placed on the burn areas 1 hour prior to seeding with the microorganisms; all excess drug was removed from the burn wound to allow the microorganisms contact with the burned area and, thereafter, the drug treatments were continued for 10 days.

Treatment of 60% burns with mafenide acetate, silver sulfadiazine and sodium chlorite-lactic acid gel. Three groups of 60% seeded burned rats were treated with single daily doses of either mafenide acetate, silver sulfadiazine or sodium chlorite-lactic acid gel. Treatments began on postburn day 0 and continued through day 10.

Toxicity studies. The standard area topically treated with graded doses of 0.6%, 0.8% and 1% sodium chlorite-lactic acid gel was 192 cm² for the 60% burn. Sodium chlorite-lactic acid gel was evenly distributed over the entire area. All treatment for the toxicity studies began on postburn day 0 and continued to postburn day 10. For comparison, groups of untreated animals were also studied. Selected rats from each test group were examined for gross and microscopic changes.

Disc susceptibility testing on graded concentrations of sodium chlorite-lactic acid gel. The concentration of sodium chlorite-lactic acid gel for this in vitro study ranged from a high of 0.8% to a low 0.2% for the determination of the susceptibility of the microorganism to the drug. Dilutions were made by using the concentrated sodium chlorite-lactic acid gel plus the addition of gelling material as a diluent. A filter paper disc 7 mm in diameter was impregnated with 0.05 ml of the appropriate concentration of sodium chlorite-lactic acid gel for agar diffusion studies. Agar diffusion studies were carried out using the standard Kirby-Bauer agar overlay technique for disc susceptibility testing.

RESULTS

Topical treatment of 20% experimental burn wound sepsis. Table 1 summarizes the results of challenges and treatment with three concentrations of sodium chlorite. One hundred sixty-one animals were burned over 20% of the total body surface and seeded with ISR strain 12-4-4 (59). These were treated daily for 10 days with sodium chlorite-lactic acid gel of varying concentrations from 0.6% to 1.0% sodium chlorite. In a group of 82 animals treated with 0.6% sodium chlorite, 36 died between days 7 and 17. A second group of 23 animals was treated with 0.8% sodium chlorite. In this group of animals, five deaths occurred between days 9 and 15. A third group of 56 animals was treated with 1.0% sodium chlorite. Three animals died between days 5 and 16. A fourth group of 59 animals burned over 20% of the total body surface and seeded with ISR strain 12-4-4 (59) served as controls. Fifty-seven died between days 4 and 12. The mortality among these four groups of animals is different, and Chi-square tests of significance show $X^2(1)$ (1,2,3 vs 4) = 83.46***, $X^2(1)$ (1 vs 2,3) = 23.11** and $X^2(1)$ (2 vs 3) = 4.80*.

Single treatment and delayed single treatment (Table 2). A group of 29 animals was burned over 20% of total body surface, seeded with ISR strain 12-4-4 (59) and treated with a single dose of sodium chlorite-lactic acid gel 1.0% on postburn day 0, followed by nine applications of placebo gel. Eighteen died between days 8 and 21. A second group of 28 animals was seeded with ISR strain 12-4-4 (59) and treated with a single dose of sodium chlorite-lactic acid gel 1.0% on postburn day 1, followed by nine applications of placebo gel. Two died between days 3 and 13. A third group of 19 animals burned over 20% of their total body surface and seeded with ISR strain 12-4-4 (59) served as burn challenge controls. Sixteen died between days 7 and 12. The mortality among these three groups of animals is different, and Chi-square test of significance shows $X^2(1)$ (1 vs 2) = 18.87**.

Table 1. Graded concentrations of sodium chlorite-lactic acid gel on 20% burn, challenged with ISR strain 12-4-4 (59)

% Sodium chlorite	Treatment (days)	No. of animals	No. died	Time to death (days)
0.6	10	82	36	7-17 (11.95)*
0.8	10	23	5	9-15 (10.4)
1.0	10	56	3	5-16 (11.0)
-	-	59	57	4-12 (9.0)

* Number in parentheses indicates the mean.

Table 2. Sodium chloride-lactic acid gel and placebo gel treatment of 20% burn challenged with ISR strain 12-4-4 (59)

% Sodium chlorite	Treatment (days)	Placebo gel	No. of animals	No. died	Time to death (days)
1.0*	1	9	29	18	8-21 (12.2)**
1.0***	1	9	28	2	3-13 (8.0)
-	-	-	19	16	7-12 (9.0)

* Single treatment on day 0 of burn and the remaining 9 days of treatment with placebo gel.

** Number in parentheses indicates the mean.

*** Single treatment on postburn day 1 and the remaining 9 days of treatment with placebo gel.

Treatment prior to seeding (Table 3). In the clinical situation, treatment of burn wounds hopefully begins prior to massive seeding of the burn wound with microorganisms. The following groups of animals demonstrate that *P. aeruginosa* can be controlled with treatment prior to seeding. One group of 20 animals was seeded over 20% of total body surface following mafenide acetate application to the burn area 1 hour prior to seeding. Single daily doses of 10% mafenide acetate continued for a period of 10 days. All animals lived for a period of 21 days of observation. A second group of 20 animals was burned over 20% of total body surface and 1% silver sulfadiazine applied to the burn area 1 hour prior to seeding. Single daily doses of 1% silver sulfadiazine continued for a period of 10 days. All animals lived for a period of 21 days of observation. A third group of 10 animals was seeded over 20% of total body surface area following sodium chlorite-lactic acid gel 1.0% application to the unseeded burn area 1 hour prior to seeding. Single daily doses of 1.0% sodium chlorite-lactic acid gel continued for 10 days. All animals lived through a period of 21 days of observation. A fourth group of eight animals was seeded over 20% of total body surface following application of 0.8% sodium chlorite-lactic acid gel to the unseeded burn area 1 hour prior to seeding with ISR strain 12-4-4 (59). One animal died on day 16. A fifth group of 18 animals burned over 20% of their total body surface and seeded with ISR strain 12-4-4 (59) served as burn challenge controls. Seventeen died between days 8 and 14.

Sixty percent burn challenge and treatment with mafenide acetate, silver sulfadiazine and sodium chlorite-lactic acid gel. Table 4 summarizes the results of treatment and challenges. The first group of 10 animals was burned over 60% of the total body surface, seeded with ISR strain 12-4-4 (59) and treated with daily doses of 10% mafenide acetate for a period of 10 days. Nine died between days 7 and 20. A second group of nine animals was burned over 60% of the total body surface, seeded with ISR strain 12-4-4 (59) and treated with daily doses of 1% silver sulfadiazine for a period of 10 days. One died on day 17. A third group of 10 animals was burned over 60% of the total body surface, seeded with ISR strain 12-4-4 (59) and treated with daily doses of 0.6% sodium chlorite-lactic acid gel. Six died between days 4 and 14. A fourth group of seven animals was burned over 60% of the total body surface and seeded with ISR strain 12-4-4 (59); these animals served as burn untreated controls. All died between days 1 and 7.

Toxicity studies (Table 5). Twenty animals were burned over 60% of the total body surface and challenged by 10 applications of 0.6% sodium chlorite-lactic acid gel. Five animals died between days 4 and 10. In a second group of 52 animals burned over 60% of the total body surface and challenged by 10 applications of 0.8% sodium chlorite-lactic acid gel, seven animals died between days 6 and 21. In a third group of 29 animals burned over 60% of their total body surface and challenged by 10 applications of 1.0% sodium chlorite-lactic acid gel, 12 animals died between days 4 and 19. Forty-three animals burned over 60% of their total body surface served as untreated burn controls. Seven animals died between days 4 and 21. The mortality among these four groups of animals differed

Table 3. Treatment of 20% burn prior to challenge with ISR strain 12-4-4 (59)

Treatment drug	Treatment (days)	No. of animals	No. died	% lived	Time to death (days)
10% mafenide acetate	10	20	0	100.0	---
1% silver sulfadiazine	10	20	0	100.0	---
1.0% sodium chlorite-lactic acid gel	10	10	0	100.0	---
0.8% sodium chlorite-lactic acid gel	10	8	1	87.5	16 (16)*
-----	--	18	17	5.5	8-14 (10.7)

* Number in parentheses indicates the mean.

Table 4. Sixty percent burn challenged with ISR strain 12-4-4 (59) treated with mafenide acetate, silver sulfadiazine, sodium chlorite-lactic acid gel

Treatment drug	Treatment (days)	No. of animals	No. died	Time to death (days)
10% mafenide acetate	10	10	9	7-20 (12)*
1.0% silver sulfadiazine	10	9	1	17 (17)
0.6% sodium chlorite-lactic acid gel	10	10	6	4-14 (9.7)
-----	--	7	7	1-7 (4)

* Number in parentheses indicates the mean.

only for the third group, and Chi-square test of significance shows $X^2_{(1)} (1,2,3 \text{ vs } 4) = 0.99\text{NS}$, $X^2_{(1)} (1,2 \text{ vs } 3) = 6.96^{**}$ and $X^2_{(1)} (1 \text{ vs } 2) = 1.38\text{NS}$.

Table 5. Toxicity studies, 60% burns treated with sodium chlorite-lactic acid gel

% Sodium chlorite	Treatment (days)	No. of animals	No. died	Time to death (days)
0.6	10	20	5	4-10 (8.4)*
0.8	10	52	7	6-21 (11.0)
1.0	10	29	12	4-19 (7.7)
-	-	43	7	4-21 (13.0)

* Number in parentheses indicates the mean.

Disc susceptibility testing on sodium chlorite-lactic acid gel (Table 6). Table 6 summarizes the in vitro disc susceptibility testing on sodium chlorite-lactic acid gel. Sixty-four isolates of microorganisms from burn patients show no evidence of resistance above 0.3% concentration of sodium chlorite-lactic acid gel. Only two isolates of Staphylococcus epidermidis were resistant in the two lowest concentrations, 0.3% and 0.2%, of sodium chlorite-lactic acid gel.

Pathology in 60% burn. Graded concentrations of sodium chlorite-lactic acid gel were tested topically daily for 10 days on 60% burned rats, and it was found that 1.0% sodium chlorite-lactic acid gel, the highest concentration used, produced significant degeneration of the liver. Cellular changes and necrosis were observed in the livers and kidneys of 11 rats that died after being treated with 1% sodium chlorite-lactic acid gel. These changes, which did not occur in the 20% burn, represent significant toxicity of sodium chlorite at this concentration. Also observed was a discoloration (bronze-tinting with hematoxylin and eosin stains) of erythrocytes in tissue sections from rats that died following topical application of sodium chlorite. Sodium chlorite, when ingested in high concentrations, is known to have a toxic effect on erythrocytes.

These toxic changes failed to appear when lower concentrations of sodium chlorite-lactic acid gel were used topically on 60% burned rats. Therefore, the concentration of sodium chlorite-lactic acid gel selected to be used for the toxicity study on 60% burns was 0.8%, applied for 10 days daily or until the animals were sacrificed for gross and pathological examinations. Forty rats were burned over 60% of their total body surface;

Table 6. Disc susceptibility testing

Microorganism	Sodium chlorite-lactic acid gel						
	0.8%	0.7%	0.6%	0.5%	0.4%	0.3%	0.2%
<i>Staphylococcus aureus</i> (av. 12 isolates)	16.09	13.74	12.00	10.88	7.93	8.89	8.25
<i>Staphylococcus epidermidis</i> (av. 2 isolates)	15.30	14.00	11.30	11.00	10.50	7.00	7.00
<i>Klebsiella pneumoniae</i> (av. 10 isolates)	13.03	12.36	12.38	9.78	8.66	8.20	7.68
<i>Enterobacter aerogenes</i> (av. 6 isolates)	13.05	13.15	12.78	9.18	9.20	8.75	7.93
<i>Enterobacter cloacae</i> (av. 10 isolates)	12.23	11.93	12.64	9.32	8.86	8.59	8.21
<i>Pseudomonas aeruginosa</i> (av. 13 isolates)	16.48	15.98	14.18	14.65	10.17	8.95	8.39
<i>Escherichia coli</i> (av. 11 isolates)	15.00	14.54	13.68	9.88	9.44	8.41	7.78

A number greater than 7 MM indicates that the organism is sensitive at that concentration.

20 rats were treated with 0.8% sodium chlorite-lactic acid gel, and the remaining 20 rats served as untreated burn controls. The treated and the untreated 60% burned rats were sacrificed for both gross and histological examinations on postburn days 6, 7, 12 and 15. The examinations showed no gross or histologic evidence of toxicity changes in the tissue studied: heart, lung, liver, kidney, spleen, adrenal and pancreas.

DISCUSSION

Although sodium hypochlorite is an effective surface bactericidal agent, it has many disadvantages and is of debatable effectiveness as a topical agent in burns. Frequent flushing of the wound is time-consuming and the use of a gelling material as a vehicle for chlorine or chlorine dioxide provides its own cover and obviates some of the objections to Dakin and Milton solutions and the envelope method.

The ideal antiseptic for burn therapy should control both gram-positive and gram-negative organisms. Unfortunately, animal models of burn wound sepsis do not exist for the study of all pathogenic microorganisms. Standardized models therefore are indispensable to assessment of such agents. Evaluation is often assisted by various in vitro methods such as disc susceptibility testing.

The results of this study show sodium chlorite-lactic acid gel to have good in vivo, as well as in vitro, properties. The compound is relatively nontoxic as shown in particular by the 60% burn toxicity study where 0.8% sodium chlorite-lactic acid gel mortality is not significantly greater than that in the control group (Table 5). Surprisingly, sodium chlorite-lactic acid gel gave excellent results with only one treatment (rather than 10) and with one day's delay (Table 2). Ordinarily, postponement of therapy makes treatment less effective. This finding probably indicates good wound penetration by the active agent. Table 4 shows sodium chlorite-lactic acid gel to be better than mafenide acetate but not as good as silver sulfadiazine in the treatment of a nonresistant virulent strain of P. aeruginosa burn wound sepsis in a 60% burn.

The sodium chlorite-lactic acid gel have to be altered before it can be used clinically. Additives such as wetting agents may make the compound work more rapidly in vitro; and raising of the pH, which is now 3.5, may reduce toxicity when high concentrations (above 0.8% sodium chlorite) of active ingredient are used.

PRESENTATIONS/PUBLICATIONS - None.

ANNEX I

FORMULAS

Part A:

Ingredient	Concentration, % w/w				
	1X	2X	3X	4X	5X
sodium chlorite	0.40	0.80	1.20	1.60	2.00
Natrosol 250 M	2.00	2.00	2.00	2.00	2.00
ethyl alcohol USP	10.00	10.00	10.00	10.00	10.00
methyl paraben USP	0.18	0.18	0.18	0.18	0.18
propyl paraben USP	0.02	0.02	0.02	0.02	0.02
purified water USP	87.40	87.00	86.60	86.20	85.80
Total	100.00	100.00	100.00	100.00	100.00

Part B:

Ingredient	Concentration, % w/w				
	1X	2X	3X	4X	5X
lactic acid, 85%	3.00	6.00	9.00	12.00	15.00
Natrosol 250 M	2.00	2.00	2.00	2.00	2.00
ethyl alcohol USP	10.00	10.00	10.00	10.00	10.00
methyl paraben USP	0.18	0.18	0.18	0.18	0.18
propyl paraben USP	0.02	0.02	0.02	0.02	0.02
purified water USP	84.80	81.80	78.80	75.80	72.80
Total	100.00	100.00	100.00	100.00	100.00

	1X	2X	3X	4X	5X
Final concentration of sodium chlorite after mixing Part A and Part B	0.2%	0.4%	0.6%	0.8%	1.0%

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DRAB(AR)436	
				DA OG 6969	81 10 01		
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DRGTH INSTN ^a	9. SPECIFIC DATA- CONTRACTOR ACCESS	10. LEVEL OF EFF A. WORK UNIT
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11. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	6T102A	3M16T102BST0		BB		302	
b. XXXXXXXX							
c. XXXXXXXX	STOG - 80	- 7.2:5					
11. TITLE (Provide with Security Classification Code) ^a							
(U) The Study of Metabolism and Nutritional Effects of Burn Injury in Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
76 10		Cont		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
Not Applicable				PRECEDENCE		20. FUNDS (in thousands)	
a. DATES/EFFECTIVE:		EXPIRATION:		FISCAL YEAR		1981	
b. NUMBER ^a				CURRENCY		6.0	
c. TYPE:		d. AMOUNT:		1982		5.5	
e. KIND OF AWARD:		f. CUM. AMT.				370	
21. RESPONSIBLE DOD ORGANIZATION				22. PERFORMING ORGANIZATION			
NAME ^a US Army Institute of Surgical Research				NAME ^a US Army Institute of Surgical Research			
ADDRESS ^a Ft Sam Houston, Texas 78234				ADDRESS ^a Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic institution)			
NAME: Basil A. Pruitt, Jr., M.D., COL, MC				NAME ^a Cleon W. Goodwin, Jr., M.D.			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2968			
23. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			

(U) Nitrogen Balance; (U) Burn Injury; (U) Computer Surveillance; (U) Metabolism;
(U) Humans; (U) Animal Model

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To identify afferent and efferent mediators of postinjury hypermetabolism and altered thermoregulation in burned soldiers. To describe the effects of thermal injury on endocrine function and metabolism of proteins, carbohydrates, and fats. To establish optimal nutritional support for thermally injured patients by computer analysis of daily balance studies.

24. (U) Environmental chambers serve as an experimental laboratory for monitoring thermoregulatory and metabolic alterations of burned patients and burned animals. Limb plethysmography is employed to measure alterations in regional blood flow of patients. An injured animal model has been developed to characterize the control of regional circulation and substrate delivery following trauma. Isolation of adipocytes from fat biopsies and arterial and venous blood analyses are conducted in both patients and animal models to measure the fluxes of various substrates from fatty depots and across different regional beds. Pertinent clinical data from daily clinical assessment and laboratory studies are stored in computerized data files for continuous on-line analysis of nutritional therapy.

25. (U) 8010 - 8109. The isolated adipocyte has been chosen as a controlled environment for determining the function of adipose tissue in normal and injured systems. Preliminary experiments have been completed

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DRAB(AR)336	
3. DATE PREV SUMRY ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9. SPECIFIC DATA- CONTRACTOR ACCESS ^a	
80 10 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ^a		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY		61102A		3H161102B510		BB 302	
b. XXXXXXXX							
c. XXXXXXXX		STOG - 80		7.2:5			
11. TITLE (Proceed with Security Classification Code) ^a							
(U) The Study of Metabolism and Nutritional Effects of Burn Injury in Soldiers (44)							
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003500 Clinical Medicine							
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76 10		Cont		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
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a. DATES/EFFECTIVE:		EXPIRATION:		FISCAL YEAR		1981	
b. NUMBER ^a				CURRENT		6.0	
c. TYPE:		d. AMOUNT:		1982		5.5	
e. KIND OF AWARD:		f. CUM. AMT.				370	
20. RESPONSIBLE S&T ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME ^a US Army Institute of Surgical Research				NAME ^a US Army Institute of Surgical Research			
ADDRESS ^a Ft Sam Houston, Texas 78234				ADDRESS ^a Surgical Study Branch Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr., M.D., COL, MC				NAME ^a Cleon W. Goodwin, Jr., M.D.			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2968			
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				NAME:			
				NAME:			
				POC: DA			
23. REVISIONS (Furnish DATE and SUMMARY CLASSIFICATION CODE)							
(U) Nitrogen Balance; (U) Burn Injury; (U) Computer Surveillance; (U) Metabolism; (U) Humans; (U) Animal Model							
24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Furnish individual paragraphs identified by number. Proceeds text of each with Security Classification Code.)							
<p>to confirm the effectiveness of this method in observing lipolytic rates in both patients and animal models. Initial values from patients for rates of triglyceride breakdown and responsiveness to hormonal stimulation are comparable to values obtained from an animal model. A continuous computer graphics program was developed to aid evaluation of the nutritional state of critically ill burn patients quickly and efficiently without having to read through and calculate long lists of chart entries. Initial assessment of metabolic expenditure and nutritional requirements of severely burned patients is calculated using nomograms stored in a computer program. The computer nomograms for predicting metabolic requirements correlate closely with physiologic data obtained by direct measurement ($r = 0.84$ for resting energy expenditure). The computer generates daily profiles of predicted calorie and protein requirements, actual intake with percentages of requirements, nitrogen/calorie ratio, weight changes, and nitrogen balance.</p>							

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

**PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF
BURN INJURY IN SOLDIERS - STUDIES OF DISTURBANCE OF
PROTEIN TURNOVER IN BURNED TROOPS: USE OF AN ANIMAL
MODEL**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**Wanda L. Brown, M.S.
Eleanor G. Bowler, Ph.M.
Arthur D. Mason, Jr., M.D.**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF
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US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1980 - 30 September 1981

Investigators: Wanda L. Brown, M.S.
Eleanor G. Bowler, Ph.M.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Groups of male Sprague-Dawley rats weighing from 180-200 g were anesthetized and subjected to a 20% body surface full-thickness scald burn or sham burn. At 1 hour postburn, rats from each group were given a subcutaneous injection of hyaluronidase (150 N.F. units in 1 ml) into the wound area. Rats from each group were sacrificed at intervals from 1-144 hours postburn. The entire wound area was rapidly excised and used for determination of water content and dry weight, or of albumin content which was determined by radioimmunoassay. Plasma volumes were determined on all of the rats. No parenteral fluids were administered.

Plasma volume fell to approximately 70% of normal during the first hour postburn but returned to normal by 24 hours postburn. Albumin and water content of wounds of untreated burned rats (BU) increased in the relative proportions in which they normally occur in plasma but the actual amounts were approximately 1.5 ml greater than the plasma volume deficit, indicating that fluid from other tissues had been translocated into the wound during that period.

The increase in water content of the burn wound (BU) reached approximately 70% of maximum at 6 hours postburn, slowed from 6-12 hours, and reached a maximum of 8.8 ml more water than sham wound at 24 hours postburn. Water content declined slowly but the burn wounds still contained 4.4 ml more water than sham wounds 144 hours postburn.

The increase in albumin content of BU also reached a maximum 24 hours postburn and remained at that level through 72 hours. The albumin content further increased slightly at 144 hours but the relative proportions of albumin in plasma and wound of BU remained constant from 1-144 hours, indicating that the two pools were in equilibrium.

Both the water and albumin content of hyaluronidase-treated burn wounds (BHY) were significantly lower than those of the wounds of BU but were higher than those of sham rats (SU and SHY). One-hour transfer rates of labeled albumin showed that this was the result of more rapid flow through the wounds of BHY, particularly during the early postburn period.

The dry weight of wounds of rats in Group BU was higher than that of rats in Group BHY. The dry weights of wounds of rats in Groups SU and SHY were significantly lower than those of both groups of burned rats. The water content and dry weight of unburned skin of burned rats (BU) were not significantly different from those of sham rats (SU).

In burned rats (BU) injected with ^{125}I -labeled rat albumin just before injury, we found that plasma albumin SA and burn wound albumin SA were equal at 1 hour postburn. In contrast, in sham rats (SU), plasma albumin SA was ten times wound albumin SA at 1 hour and SA in the two pools did not become equal until 24 hours postburn. Subsequently, the plasma albumin SA curve of BU declined at approximately the same rate as the plasma and wound albumin SA of sham rats (SU). BU wound albumin SA continued higher than the others through 144 hours postburn. In hyaluronidase-treated burned rats the rate of decrease of albumin SA in plasma and wound (BHY) was more rapid than from those pools in Group BU during the first 72 hours postburn but the rate slowly declined.

The labeled albumin data, when calculated as the percent of dose remaining in plasma, showed that almost twice as much labeled albumin disappeared from plasma of burned rats (BU) during the first hour postburn and that 85% of that loss entered the burn wound. In contrast, 3% of labeled albumin loss from sham plasma (SU) entered the sham wound in 1 hour.

We believe that due to the changes in the physical properties of the connective tissue of the interstitium, to the large increase in the burn wound albumin pool size, and to the altered hourly exchange rates of labeled albumin in the burn wound, the burn wound is an additional extravascular compartment which cannot be satisfactorily described by the simpler mathematical models which have customarily been used in the past. These changes also are the cause of the persistent edema in the burn wound.

Albumin
Labeled albumin
Rat
Burn
Edema
Hyaluronidase

STUDIES OF DISTURBANCE OF PROTEIN TURNOVER IN BURNED TROOPS: USE OF AN ANIMAL MODEL

INTRODUCTION

In a previous study of the incorporation of (2-¹⁴C)glycine into serum proteins of rats on the sixth day postburn, we concluded that the prolonged hypoalbuminemia which occurs following burn injury is not caused by impaired synthesis but is, instead, a consequence of altered compartmentation (1). The postburn decrease in plasma albumin pool size could be accounted for by an increase in the wound albumin pool, while other tissues showed no change. Because albumin isolated from the burn wound on the sixth postburn day had the same properties as native albumin we concluded that the wound albumin pool was not static, but that it continued to exchange with the plasma albumin pool.

Our purpose in this study was to extend our previous work to the early postburn period to determine the time course of edema development using the same experimental rat burn model and the same analytical procedures used in that study. To accomplish this we have made direct measurements of albumin in plasma and in burn wound, of water content in burn wound, and of labeled albumin exchange in rats with a 20% body surface burn between 1 hour and 6 days postburn. In addition we have measured the effect on edema accumulation of injecting hyaluronidase into the burn wound.

ANIMAL MODELS

Young Sprague-Dawley rats (Holzman, Madison, Wisconsin) weighing 180-200 g were anesthetized with sodium pentobarbital (1 mg/25 g) intraperitoneally. The hair on the dorsum was clipped and the rats were placed in a protective mold which limited the area to be burned to 20% of the body surface. Immersion of the exposed area of the dorsum in boiling water for 10 seconds produced a full-thickness burn with sharp margins (2).

Rats for sham burn were anesthetized, clipped, placed in the protective mold, and an area on the dorsum equal to that of the burn area was outlined in ink.

At 1 hour postburn some rats from each group were given subcutaneous injections of 0.2 ml hyaluronidase (Wydase, Wyeth Laboratories, Inc., Philadelphia) in 0.15 M NaCl into each of five sites in the wound (total dose was 150 N.F. units in 1 ml). Other rats injected in the same manner with 0.15 M NaCl served as controls for total water determination. The water content of the wounds of the saline control group proved not to be

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1. Brown WL, Bowler EG, Mason AD Jr, Pruitt BA Jr: Protein metabolism in burned rats. *Am J Physiol* 231:476-482, 1976.
 2. Walker HL, Mason AD Jr: A standard animal burn. *J Trauma* 8:1049-1951, 1968.

different from that of the untreated rats so, for brevity, those results are not shown. This leaves the following four groups:

Group SU: Sham untreated
Group SHY: Sham hyaluronidase-treated
Group BU: Burned untreated
Group BHY: Burned hyaluronidase-treated

The rats were housed in individual cages and were permitted free access to food (Purina Lab Chow) and water. No parenteral fluids were administered.

MATERIALS AND METHODS

The following procedures have been previously described in detail (1). Briefly, approximately 5 minutes before the scheduled time of sacrifice, 0.5 ml (0.4 μ Ci) of 131 I-labeled human serum albumin (Mallinckrodt, Hazelwood, Missouri) was injected into the tail vein of each rat. Each rat was anesthetized with methoxyflurane, and as much blood as possible was withdrawn from the heart. Care was taken to obtain a sample within 3-6 minutes for determination of plasma volume by isotope dilution.

The tissue within the margins of the burn wound, or within the inked outline of the sham wound, was rapidly excised through the panniculus carnosus to fascia. The entire sample, approximately 68 cm² surface area, was used for analysis. This sample will be referred to below as wound tissue whether from burned or sham burned rats.

The remainder of the skin (unburned skin) was removed from some of the untreated rats for determination of total water content and dry weight.

Total Water Determination

Tissue samples were weighed immediately after excision and were dried to constant weight at 70° C. Total water was determined as the difference between the wet and dry weights.

Albumin Determination

The tissue from the wound was weighed, minced, homogenized in 9 volumes of 0.1% deoxycholate in 0.15 M NaCl, pH 8.0, and centrifuged (all at 4° C). Aliquots of the supernate and of plasma were immediately frozen and stored at -20° C until the time of analysis. Albumin content of extracts and of plasma was determined by radioimmunoassay.

Rat albumin, isolated by an alcohol-trichloroacetic acid extraction procedure (3) from freshly drawn normal rat serum, was labeled with 125 I

3. Katz J, Sellers AL, Bonorris G, Goldon S. Studies on the extravascular albumin of rats. In Plasma Protein Metabolism - Regulation of Synthesis, Distribution and Degradation, M.A. Rothschild and T. Waldmann, Eds., Academic Press, New York, 1970, pp 135-136.

(carrier free, New England Nuclear, Boston) by an iodine monochloride procedure (4). Average specific activity of the preparation was 80-100 $\mu\text{Ci}/\text{mg}$ albumin and it contained less than 1% free ^{125}I .

- 1) Rats were injected with ^{125}I labeled albumin at various times postburn (0.4 $\mu\text{Ci}/\text{rat}$) and were sacrificed 1 hour after the injection.
- 2) Labeled albumin was injected (8 $\mu\text{Ci}/\text{rat}$) just before burn or sham burn and the animals were sacrificed at intervals from 1 hour to 144 hours postburn.
- 3) Two sham rats (SU) and four burned rats (BU) were injected with labeled albumin (8 $\mu\text{Ci}/\text{rat}$) at 24 hours postburn and sacrificed at 144 hours postburn.

Plasma volume and total plasma and wound albumin determinations were performed on each of the rats in addition to labeled albumin determinations.

Corrections for intravascular albumin retained in the tissues was made from the ratio of the ^{131}I specific activities of tissue and plasma albumin. This was a particularly important correction in the 1-hour transfer studies. In addition, for those rats which were injected with labeled albumin during the first few hours postburn when the burned rats' plasma volumes were significantly lower than those of the sham rats, the initial specific activities of the albumin were adjusted to make them comparable to those of the sham rats, using the ratio of the plasma albumin pool sizes of burned and sham rats.

Tissue extracts and diluted plasma samples were precipitated with an equal volume of 20% trichloroacetic acid (TCA) to separate protein-bound and unbound iodine. ^{125}I and ^{131}I contents of the samples were determined using a two-channel automatic gamma counter set to discriminate between the two isotopes. Counting efficiency for ^{125}I was 67% and for ^{131}I was 40%. Statistical counting error for all samples was 2-5%.

Statistical Procedures

The significance of the differences between the treatment groups (other than for the tracer studies) was determined by analysis of variance (ANOV) using a computer program implementing a procedure outlined by Steel and Torrie (5). This program permitted comparison, with or without transformation of data, between groups of unequal size. Where noted, the data were transformed to Napierian logarithms (\ln) before analysis to minimize

4. McFarlane AS: In vivo behavior of ^{131}I -fibrinogen. J Clin Invest 42:346-361, 1963.

5. Steel RGD, Torrie JH: Principles and Procedures of Statistics. McGraw Hill, New York, pp 112-115.

heterogeneity of variance (6). Slopes and intercepts of labeled albumin disappearance curves were determined by analysis of covariance (ANOCOV) of \ln (specific activity or percent of dose) versus t (hour). Independent comparisons between treatment groups were: SU versus SHY; BU versus BHY; and (SU + SHY) versus (BU + BHY).

RESULTS

Plasma Volume

The mean plasma volume of burned rats was 3.2 ml less than that of sham rats at 1 hour postburn and remained at approximately that volume for 6 hours (Table 1).

At 24 hours postburn, the mean plasma volume of rats in Group BHY showed a small, but statistically significant, increase over that of rats in Group BU. Afterwards, the mean plasma volumes of rats in Groups BU and BHY were not significantly different.

The mean plasma volumes of sham rats (SU and SHY) were significantly different from one another only at 72 and 144 hours postburn. However, the plasma volumes of rats in both sham groups were within normal range throughout the period of study.

Mean plasma volumes of burned rats (BU + BHY) were smaller than those of sham rats (SU + SHY) at 24 hours postburn but were greater than those of sham rats at 48 hours postburn.

Total Water in Wound

The rate of increase in water content of the wounds of the burned rats was greatest during the first half-hour postburn (Fig 1). Thirty minutes after injury the wounds of rats of Group BU contained a mean of 3.2 ml more water than did the wounds of rats of Group SU. At 1 hour postburn the excess water content of the wounds of burned rats (BU) had increased to 4.4 ml. This was approximately 1.7 ml greater than the decrease in the plasma volume (2.7 ml) which occurred during that period, indicating that fluid had been translocated from other tissues through the plasma into the wound.

The water content of the wounds of rats in Group BU continued to rise and reached a mean of 6.1 ml more than sham wounds at 6 hours postburn. The rate of increase slowed between 6 and 12 hours postburn before it again began to rise. Maximum water content was attained at 24 hours postburn, at which time the wounds of rats in Group BU contained a mean of 8.8 ml more water than did the wounds of rats in Group SU. Although the water content of the burn wounds (BU) declined after 24 hours postburn, at 144 hours postburn the water content remained approximately 1.8 times that of sham wounds (SU).

6. Bartlett MS, Kendall DG. The statistical analysis of variance - heterogeneity and the logarithmic transformation. J Royal Stat Soc Suppl 8:128-138, 1946.

Table 1. Plasma Volume

Hour post-burn	ml plasma per 100 g rat weight				ANOVA comparisons, p =			
	SU	SHY	BU	BHY	SU vs SHY	BU vs BHY	SU vs (SU + SHY)	vs (BU + BHY)
1	4.20 ± 0.13 (3)	-	2.83 ± 0.01 (3)	-				
3	3.89 ± 0.12 (4)	3.69 ± 0.10 (5)	2.68 ± 0.05 (4)	2.81 ± 0.07 (3)	NS	NS		< 0.001
4	4.13 ± 0.05 (3)	4.04 ± 0.07 (3)	2.85 ± 0.10 (5)	3.09 ± 0.26 (5)	NS	NS		< 0.001
24	4.34 ± 0.09 (11)	4.19 ± 0.09 (7)	3.87 ± 0.07 (26)	4.23 ± 0.09 (20)	NS	< 0.001	< 0.01	
48	4.09 ± 0.12 (6)	4.31 ± 0.05 (7)	4.36 ± 0.06 (12)	4.51 ± 0.07 (15)	NS	NS		< 0.05
72	4.31 ± 0.06 (3)	3.85 ± 0.10 (9)	4.14 ± 0.13 (9)	4.15 ± 0.09 (10)	< 0.05	NS		NS
144	4.25 ± 0.01 (4)	3.72 ± 0.08 (10)	3.89 ± 0.11 (17)	3.73 ± 0.08 (10)	< 0.05	NS		NS

TOTAL WATER IN WOUND

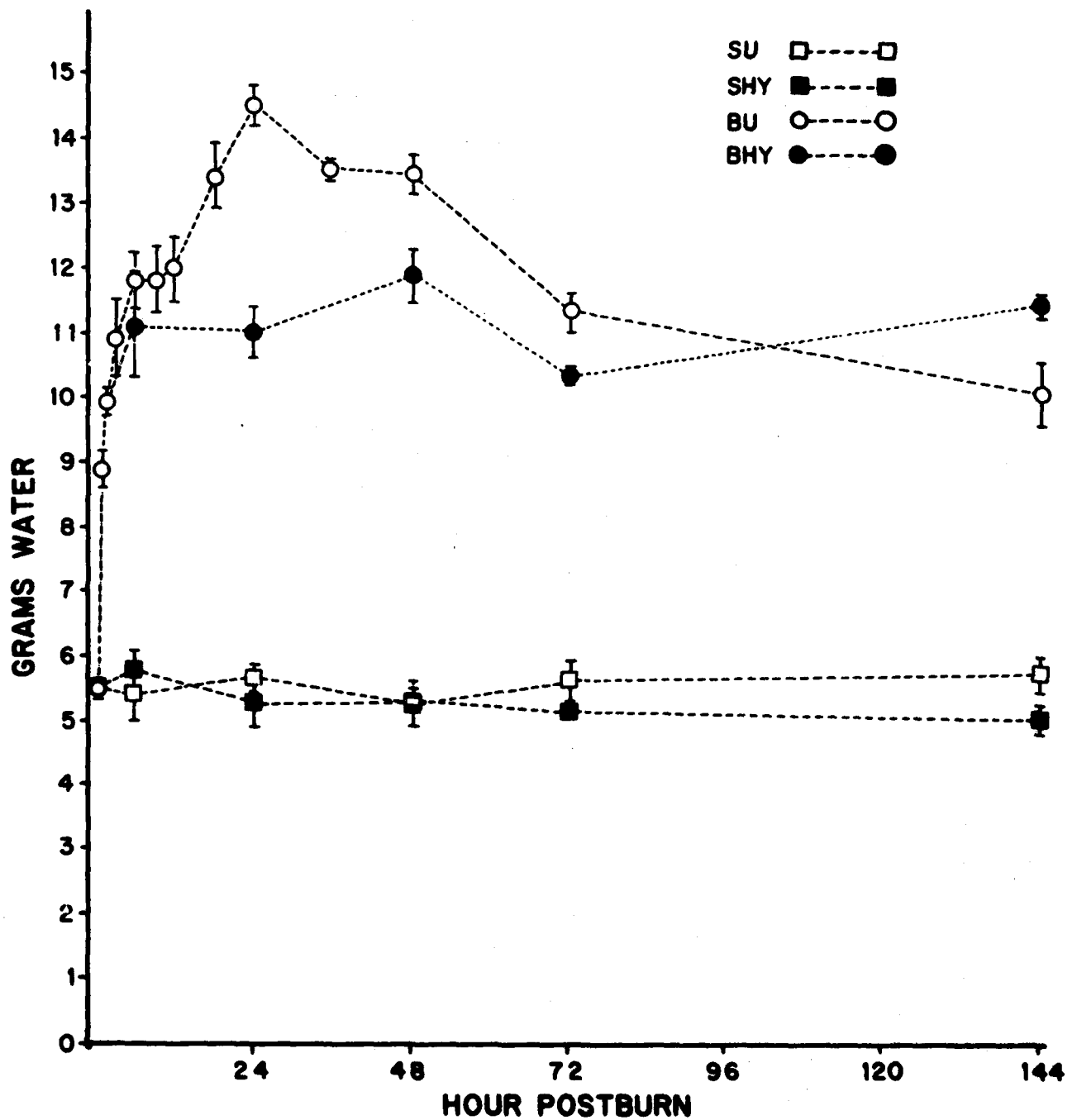


Figure 1

The mean water content of the wounds of rats in Group BHY increased very little after 6 hours postburn. It was significantly lower than that of wounds of rats in Group BU at 24 hours ($p < 0.001$), 48 hours ($p < 0.01$), and 72 hours ($p < 0.05$) postburn.

The mean water contents of wounds of sham rats (SU and SHY) were similar except at 144 hours postburn when the wounds of rats in Group SHY showed a small, but statistically significant, decrease in water content ($p < 0.05$).

Mean water contents of wounds of burned rats (BU + BHY) were significantly higher than those of sham rats (SU + SHY) at each time measured ($p < 0.001$).

Dry Weight of Wound

The mean dry weight of wounds of rats in Group BHY was significantly lower than that of rats in Group BU at 24 and 72 hours postburn ($p < 0.05$) but not significantly different at other times (Fig 2).

The mean dry weight of wounds of rats in Group SHY was significantly lower than that of Group SU at 72 hours ($p < 0.05$) and at 144 hours ($p < 0.01$) postburn, but they were similar at the other times.

Mean dry weights of wounds of burned rats (BU + BHY) were significantly higher than those of sham rats (SU + SHY) at each time measured ($p < 0.001$).

Water Content and Dry Weight of Unburned Skin

The mean water content of unburned skin of rats in Group BU was higher than that of the rats in Group SU at 1/2, 18, and 36 hours (Fig 3). Although these differences were statistically significant ($p < 0.05$), the volume changes were small. At 24 hours postburn, the time at which the mean water content of the burn wound of rats in Group BU reached a maximum, the mean water content of the unburned skin of those rats dropped sharply, but it was still within the lower limits of the control values.

The mean dry weight of the unburned skin of burned rats (BU) was significantly lower than that of sham rats (SU) at 24 hours postburn ($p < 0.01$) but was not significantly different at other times.

Total Plasma Albumin

The mean total plasma albumin of rats in Group BU was approximately 60% of that of rats in Group SU at 1 hour postburn (Fig 4).

Total plasma albumin of rats in Group BHY was greater than that of rats in Group BU at 3 hours postburn ($p < 0.001$). Total plasma albumin of burned rats (BU and BHY) was not significantly different from 24 to 72 hours postburn. At 144 hours postburn, the total plasma albumin of rats in Group BHY was significantly lower than in those in Group BU ($p < 0.01$).

DRY WEIGHT WOUND

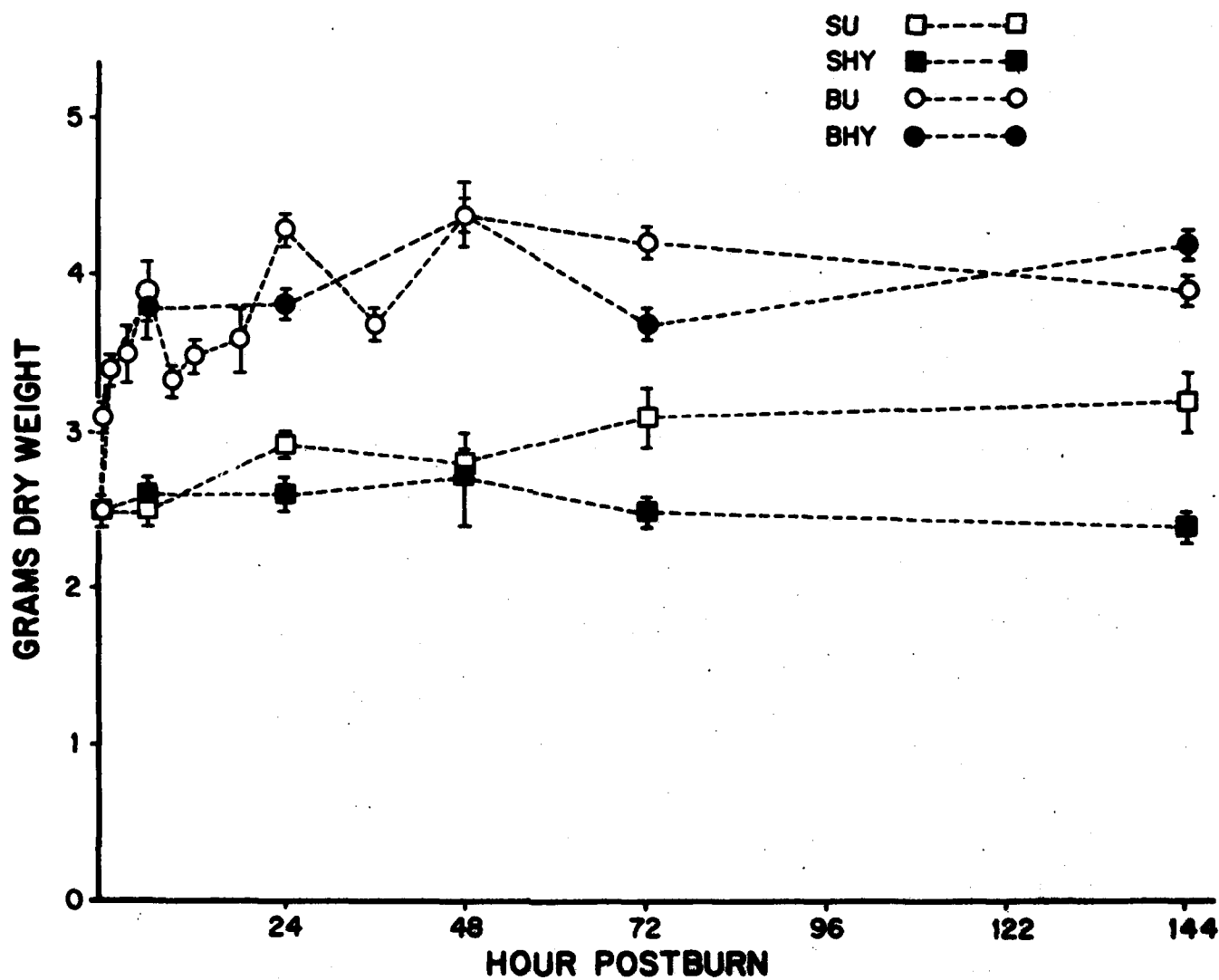


Figure 2

UNBURNED SKIN

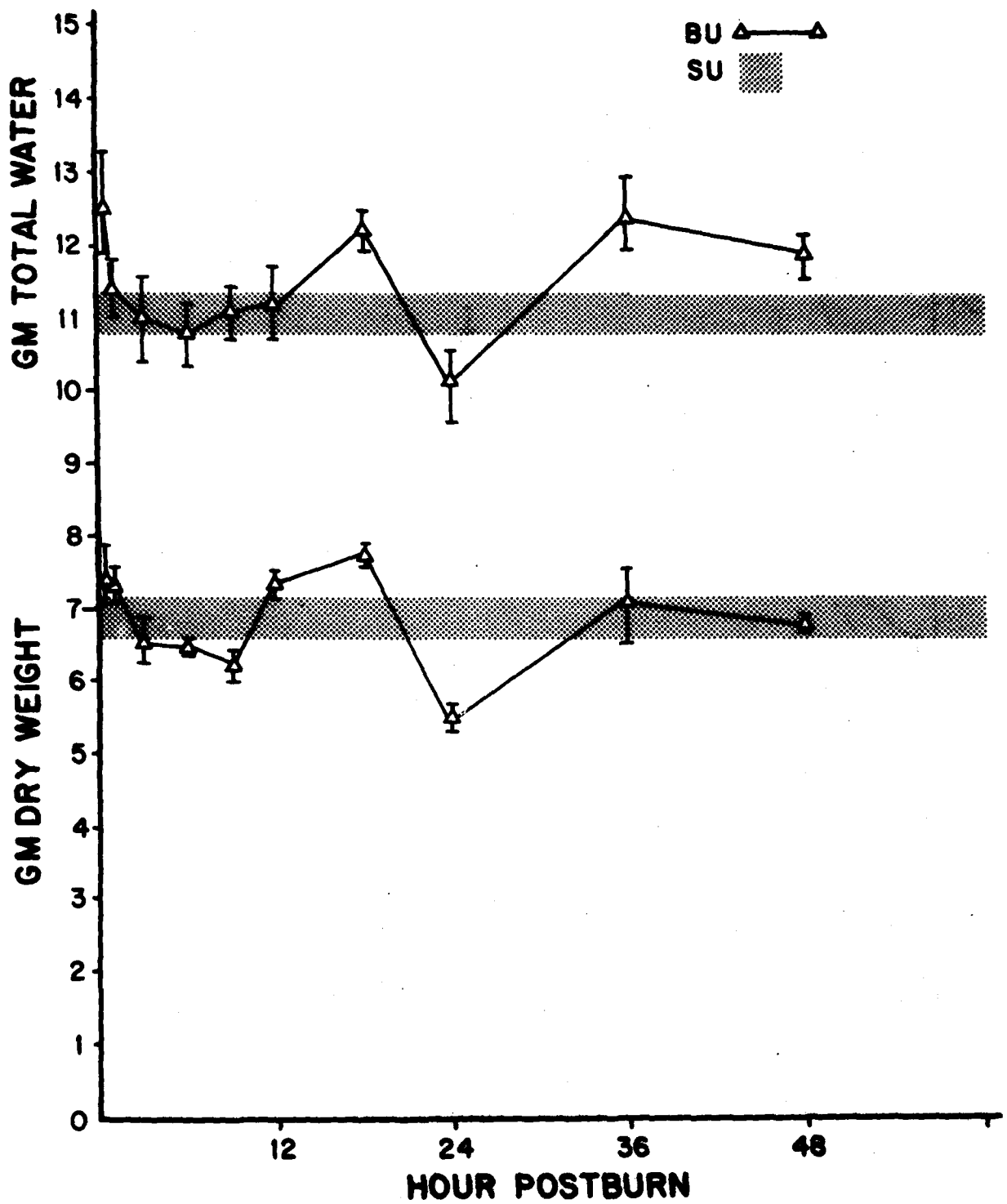


Figure 3

TOTAL PLASMA ALBUMIN

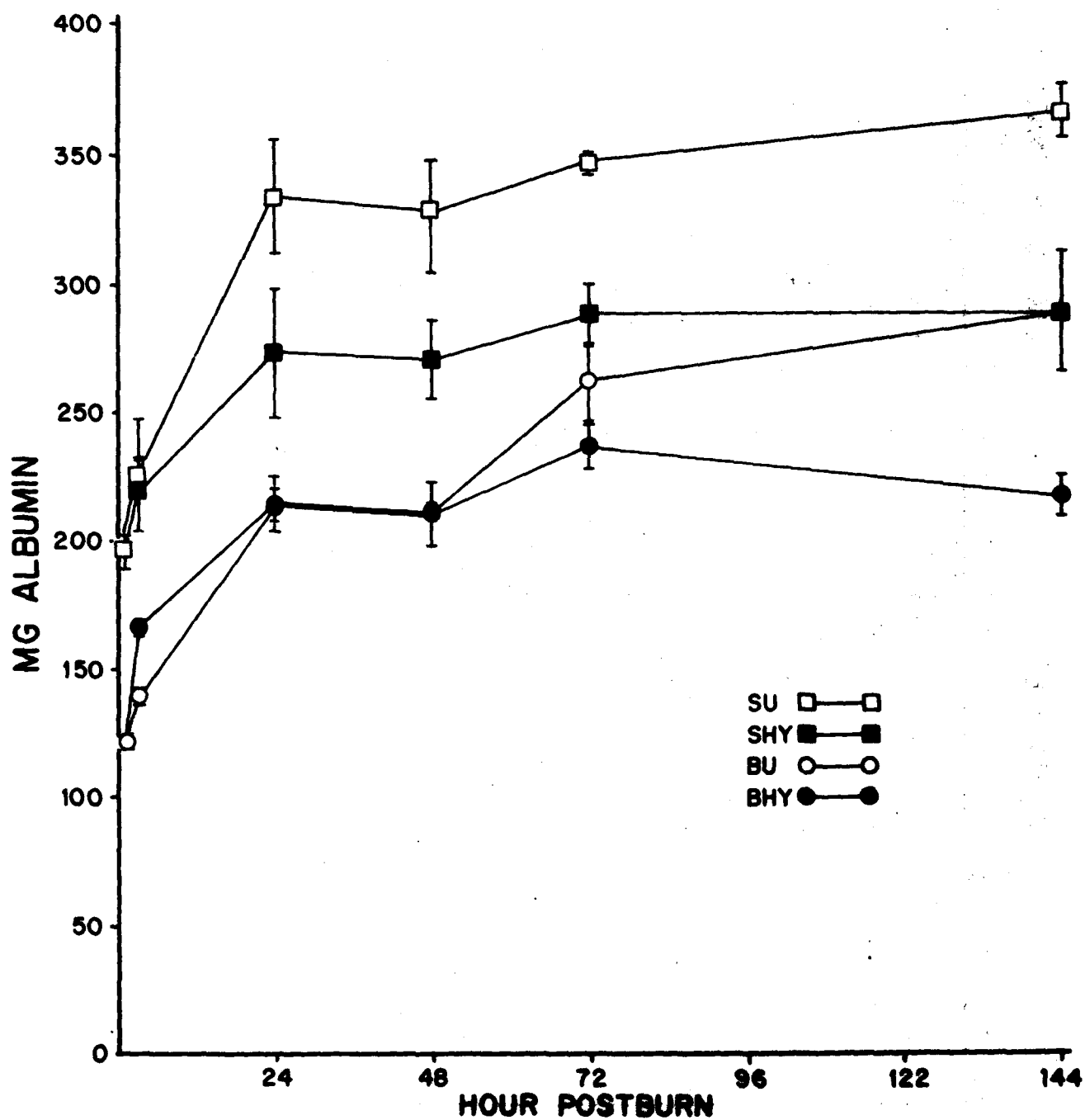


Figure 4

The mean total plasma albumin of rats in Group SHY was significantly lower than that of rats in Group SU from 24-144 hours postburn ($p < 0.05$).

Mean total albumin of burned rats (BU + BHY) was significantly lower than that of sham rats (SU + SHY) at each time measured (3-72 hours, $p < 0.001$; 144 hours, $p < 0.01$).

Total Albumin in Wound

The mean albumin content of wounds of rats in Group BU was four times that of wounds of rats in Group SU at 1 hour postburn (Fig 5). This increase in the wound albumin content was approximately 26 mg greater than the decrease in the plasma albumin content during that period, indicating that translocation of albumin as well as water from other tissues had occurred.

The mean albumin content of wounds of rats in Group BHY showed a small, but statistically significant, increase over that of wounds of rats in Group BU at 3 hours postburn ($p < 0.01$), but was significantly lower than that of Group BU from 24-72 hours postburn ($p < 0.001$). There was no significant difference in albumin content of wounds of rats in Groups BU and BHY at 144 hours postburn.

Mean albumin content of wounds of rats in Group SHY was significantly higher at 3 hours postburn ($p < 0.01$), and significantly lower at 48 hours ($p < 0.05$) and 72-144 hours postburn ($p < 0.001$) than that of rats in Group SU.

Total plasma albumin of sham rats (SU + SHY) was significantly higher ($p < 0.001$), and the wound albumin content significantly lower ($p < 0.001$) than that of burned rats (BU + BHY) at each time measured.

^{125}I -labeled Albumin Injected 1 Hour Pre-kill

After the first hour postburn, the 1-hour rate of disappearance of labeled albumin from plasma of burned rats (BU and BHY) decreased rapidly. After 24 hours postburn, the 1-hour disappearance rate from plasma was similar for all four groups of rats (Fig 6).

No significant differences were observed between the mean rates of entry (% dose/hr) of ^{125}I -labeled albumin into the hyaluronidase-treated wounds of sham rats (SHY) and untreated sham wounds (SU).

The mean rate of entry of labeled albumin into the hyaluronidase-treated burn wounds (BHY) remained higher than that into untreated burn wounds (BU) through 24 hours postburn ($p < 0.05$), but was significantly lower than that into untreated wounds at 48 and 72 hours ($p < 0.01$).

The mean rates of entry of labeled albumin into wounds of rats in Groups (BU + BHY) were significantly higher than those into sham wounds Groups (SU + SHY) at each time measured ($p < 0.001$).

TOTAL ALBUMIN IN WOUND

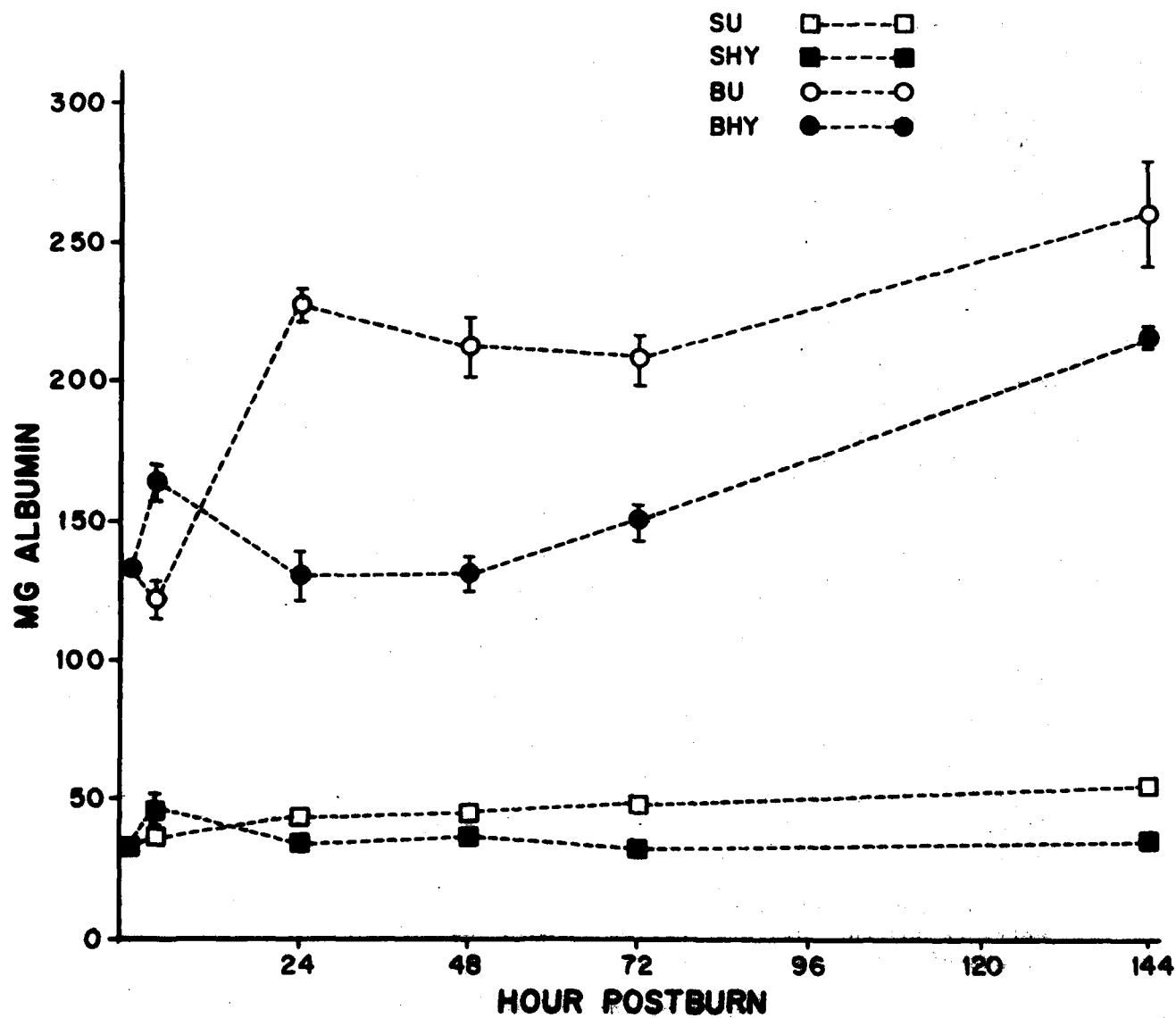


Figure 5

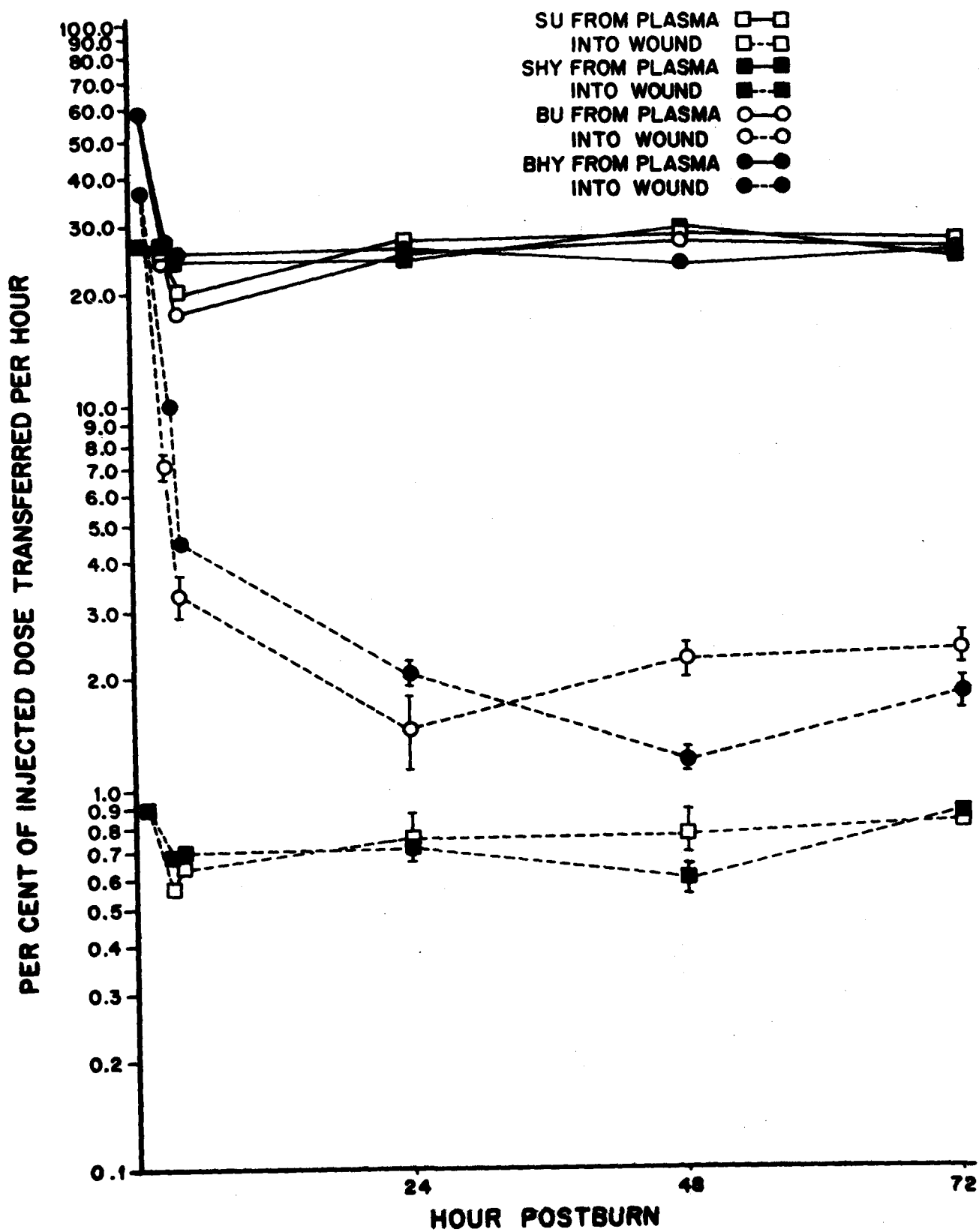


Figure 6

The quantity of albumin transferred from plasma into wound (mg/24 hr) was estimated by multiplying the quantity of total albumin in the plasma pool by the percentage transfer rate/hour of labeled albumin into the wound. This showed that approximately 100 mg albumin was transferred into the wounds of rats in Group BU, and 90 mg into wounds of rats in Group BHY each 24 hours over the period from 24-72 hours postburn. The value for sham rats (SU and SHY) was approximately 50 mg/24 hours.

In spite of the accelerated transfer of labeled albumin into wounds of burned rats, the total albumin content of the wounds remained approximately 200 mg for Group BU and 150 mg for Group BHY during the period 24-72 hours postburn, indicating that an equal quantity of albumin was being returned to plasma from the wound. Thus, the burn wound albumin pool was not static, but was in dynamic equilibrium with the plasma pool.

Specific Activity - ^{125}I -labeled Albumin Injected Preburn

The mean specific activities (SA) of plasma albumin from sham (SU) and burned (BU) rats were not significantly different at 1 hour postburn (Fig 7). However, the mean wound albumin SA of the burned rats was ten times that of the sham rats and was equal to approximately 80% of plasma albumin SA of rats in Group BU by that time.

Analysis of covariance (ANOCOV) of the 24-144 hour segment of the plasma albumin SA curves showed that the curves of all four groups of rats (Figs 7 and 8) had common slopes and intercepts. The best fit equation was:

$$y = 11292e^{-0.0164t}, r^2 = 0.96$$

ANOCOV of the 24-144 hour segments of the wound albumin SA curves showed that Groups SU and SHY had common slopes and intercepts, as did those of Groups BU and BHY. The best fit equations were:

$$\text{Groups SU and SHY: } y = 12375e^{-0.0151t}, r^2 = 0.96$$

$$\text{Groups BU and BHY: } y = 18580e^{-0.0121t}, r^2 = 0.90$$

In spite of the reasonably good fit of the curves for burned rats, it should be noted that the plasma albumin SA and the wound albumin SA changed very little between 24 and 48 hours in contrast to the curves of the sham and hyaluronidase-treated burn groups which had steeper slopes during that period.

The slopes of the wound albumin SA curves of sham rats (SU + SHY) were significantly higher ($p < 0.05$) and the intercepts were significantly lower ($p < 0.001$) than those of burned rats (BU + BHY).

The slopes of the plasma and wound albumin SA of rats in Groups SU and SHY were not significantly different between 24 and 144 hours postburn. The intercept of the wound albumin SA curves was only a little higher than

SPECIFIC ACTIVITY

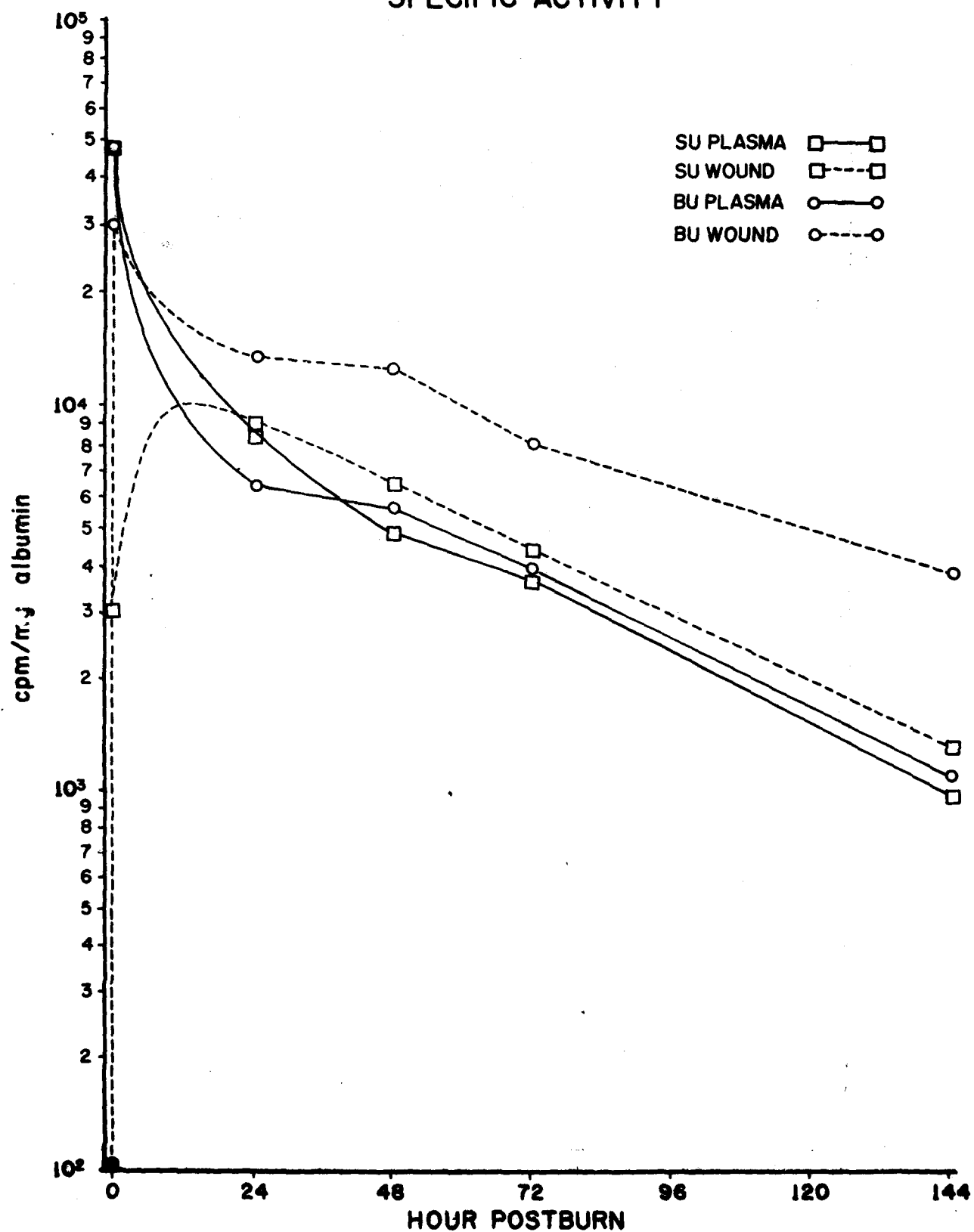


Figure 7. ^{125}I -labeled albumin injected preburn.

SPECIFIC ACTIVITY

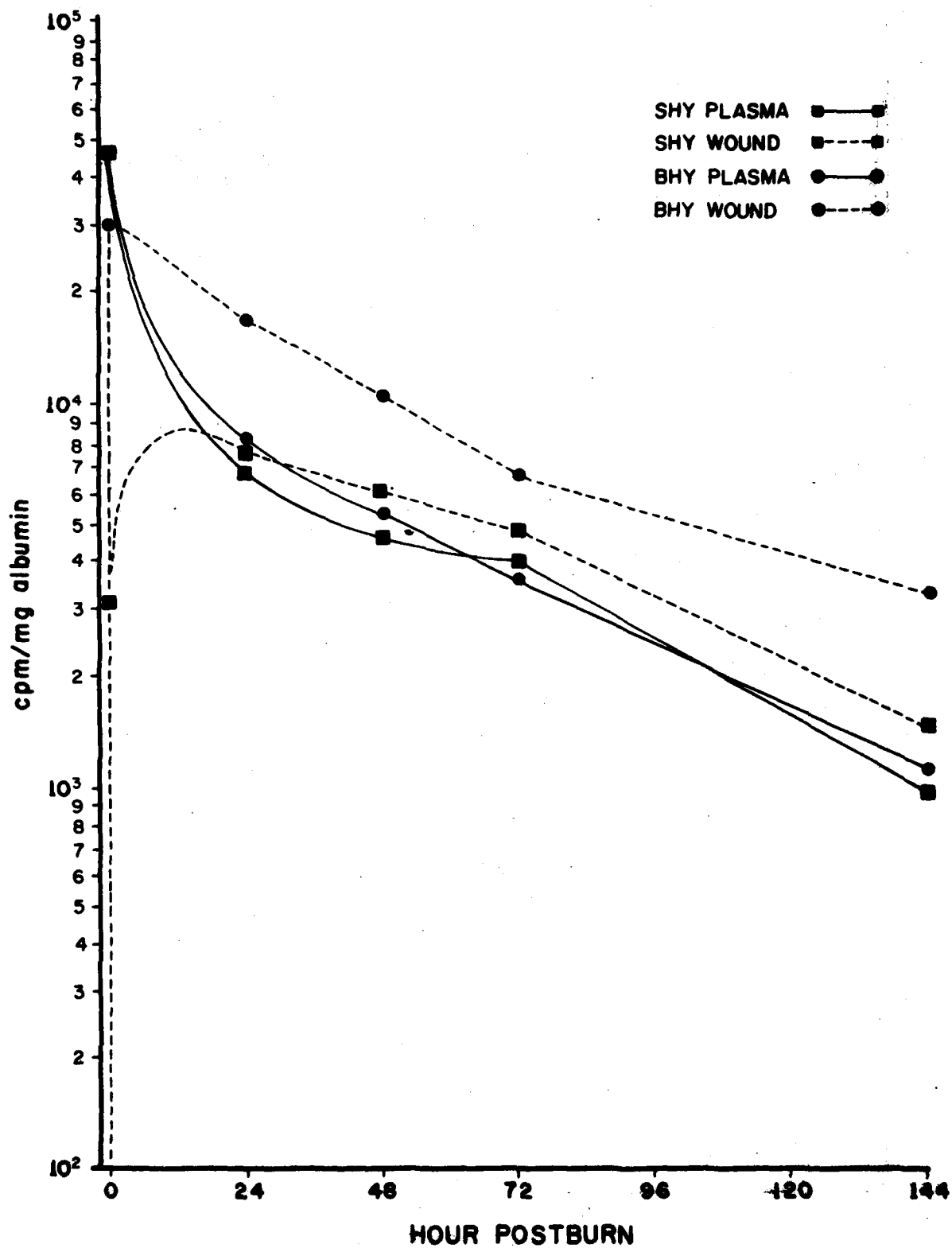


Figure 8. ^{125}I -labeled albumin injected preburn.

that of plasma albumin SA, but the differences were statistically significant (SU, $p < 0.01$; SHY, $p < 0.05$).

Both the slopes and the intercepts of the wound albumin SA curves of rats in Groups BU and BHY were higher than those of the plasma albumin SA curves. The differences were statistically significant (slopes: BU, $p < 0.01$; BHY, $p < 0.05$; intercepts: BU and BHY, $p < 0.001$).

^{125}I -labeled Albumin Injected at 24 Hours Postburn

The mean SA of plasma and wound albumin of two sham rats (SU) injected at 24 hours postburn and sacrificed at 5 days after injection were slightly different (plasma SA, 2168; wound SA, 3133). Mean wound albumin SA of four burned rats (BU) was 2.5 times plasma albumin SA at that time (plasma SA, 1842; wound SA, 4551).

Mean percent of dose remaining at 5 days after the injection in sham rats (SU) was: plasma, 6.9%; wound, 1.6%. For burned rats (BU) the values were: plasma, 5.0%; wound, 12.3%.

Percent of Dose - ^{125}I -labeled Albumin Injected Preburn

When the disappearance data were plotted as $\log (\% \text{ dose})$ vs t (hour) (Figs 9 and 10) the shape of the curves contrasted sharply with those of the specific activity (SA) curves. Whereas the plasma albumin SA of rats in Groups BU and SU were not significantly different at 1 hour postburn, the data calculated as percent of dose showed that almost twice as much of the labeled albumin had disappeared from the plasma of rats in Group BU as from the plasma of Group SU during that hour and that approximately 85% of the quantity of labeled albumin lost from the plasma of rats in Group BU was present in the burn wound at 1 hour postburn. Only 3% of the labeled albumin lost from the plasma of rats in Group SU was present in the sham wound at that time.

ANOCOV of the $\ln (\% \text{ dose in plasma})$ vs t (hour) curves (24-144 hour segment) showed that the slopes (fractional disappearance rates) of the curves of Groups SU and SHY were not significantly different, but the intercept (labeled albumin content) of the plasma albumin curve of Group SU was significantly higher than that of Group SHY ($p < 0.001$). The best fit equations were:

$$\text{Group SU: } y = 40.41e^{-0.0179t}, r^2 = 0.99$$

$$\text{Group SHY: } y = 30.33e^{-0.0169t}, r^2 = 0.98$$

The disappearance curves for labeled albumin in plasma of Groups BU and BHY had common slopes and intercepts. The best fit equation was:

$$\text{Group BU and BHY: } y = 20.74e^{-0.0147t}, r^2 = 0.95$$

The intercepts of plasma labeled albumin disappearance curves of sham rats (SU + SHY) were significantly higher ($p < 0.001$) than those of burned

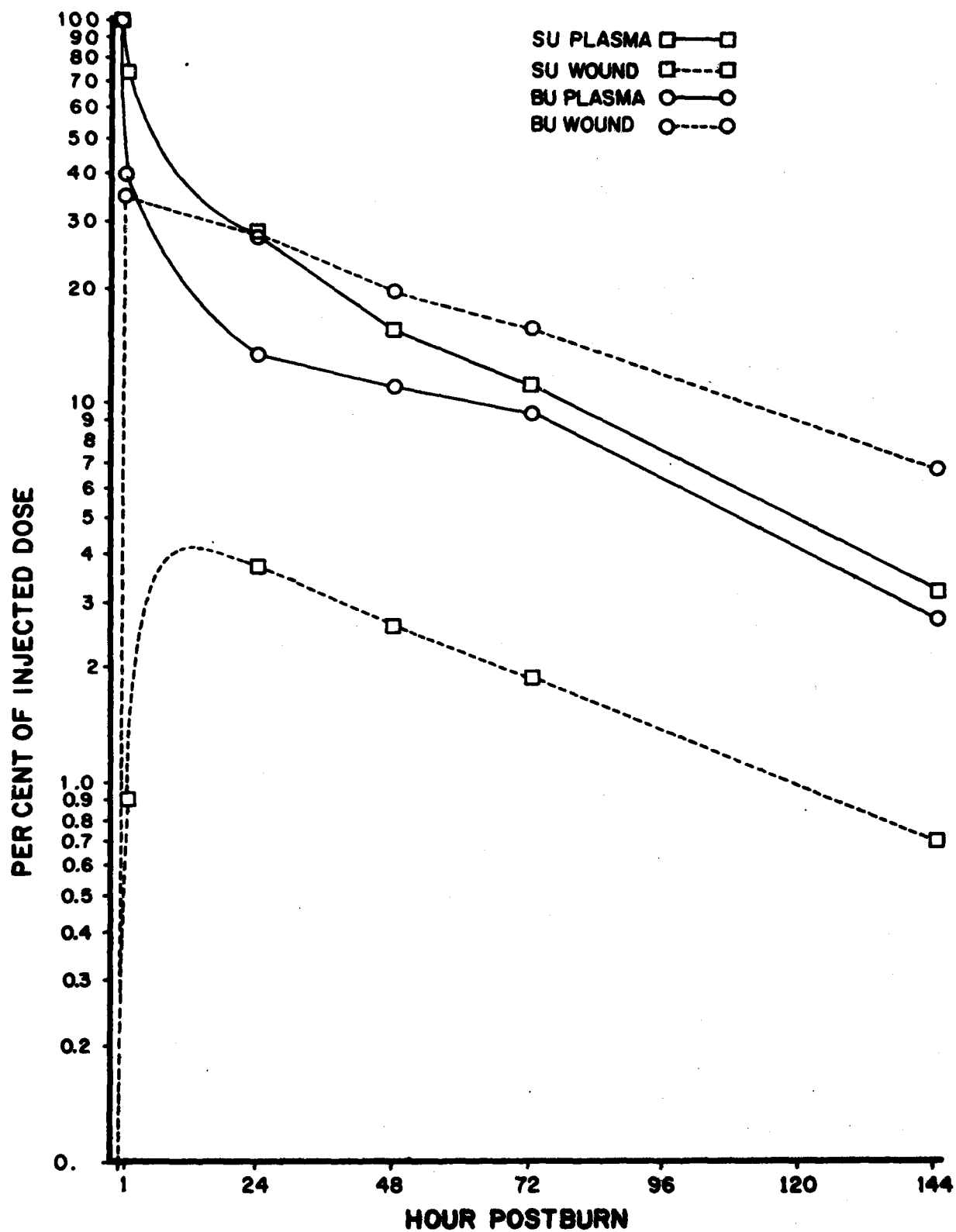


Figure 9. ^{125}I -labeled albumin injected preburn.

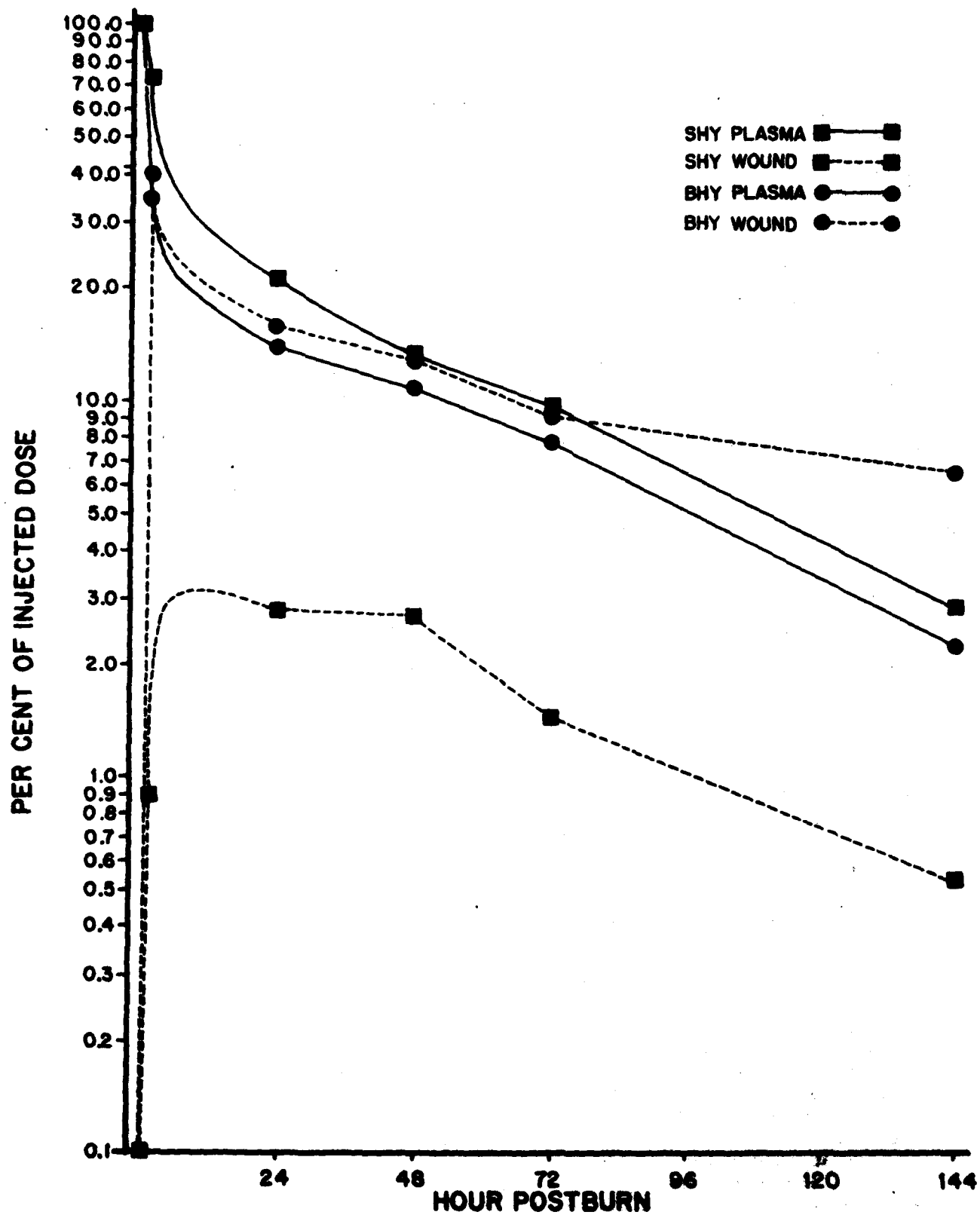


Figure 10. ^{125}I -labeled albumin injected preburn.

rats (BU + BHY), indicating that transfer from plasma was more rapid in the burned rats. The differences in the slopes of the plasma curves are difficult to interpret because of the large amount of labeled albumin accumulated in the burn wound immediately postburn; this highly labeled albumin returning to the plasma may have masked the loss of labeled albumin from the plasma of the burned rats.

The slopes of the percent dose curves for wounds of sham rats were not significantly different but the intercept for the curve of Group SU was significantly higher than that of Group SHY ($p < 0.01$). The equations were:

$$\text{Group SU: } y = 5.11e^{-0.0138t}, r^2 = 0.98$$

$$\text{Group SHY: } y = 4.21e^{-0.0158t}, r^2 = 0.94$$

The curves for the disappearance of labeled albumin from wounds of burned rats (Groups BU and BHY) were parallel from 24-72 hours postburn. Afterwards, the slope of the curve for rats in Group BHY flattened while the curve of Group BU continued downward. As a consequence, the percent of dose of labeled albumin remaining in the wounds of the burned rats (BU and BHY) was almost equal at 144 hours postburn.

In an attempt to improve the fit of the curve for burn wound albumin disappearance, an ANOCOV including only the data points for the interval from 24-72 hours postburn was performed. Even so, the fit was much less precise than that of the other curves. The best fit equations were:

$$\text{Group BU: } y = 35.40e^{-0.0116t}, r^2 = 0.88$$

$$\text{Group BHY: } y = 21.48e^{-0.0133t}, r^2 = 0.69$$

The intercept of the wound albumin curve of Group BU was significantly higher than that of Group BHY ($p < 0.001$); the slopes were not significantly different.

The intercepts of the wound albumin curves of sham rats (SU + SHY) were significantly lower ($p < 0.0001$) and the slopes were significantly higher ($p < 0.001$) than those of burned rats (BU + BHY).

DISCUSSION

The results reported here cannot be directly compared with those of other investigators of edema in burn injury because of differences in experimental models and procedures. In most studies of burn edema it has been customary to report the water content of tissues as percentages, or as the quantity per gram fat-free dry weight, in an attempt to compensate for the changes in wet weight of the burned tissues. When such values are used as the basis for comparison of burned with normal tissues, error is introduced because the dry weight and fat content, as well as the water content, vary with time after injury. Because of the marked swelling of the burned tissue it is difficult to obtain comparable samples from burned and sham wounds using biopsy techniques. It was for these reasons that we

chose to use the area of tissue delineated by the margins of the opening in the burning mold (at the time of injury) as the unit for comparison of changes in albumin and water content.

Edema development in the rat burn wound (BU) reaches 70% of maximum after 6 hours and is maximal at 24 hours postburn, a course similar to that in the human burn wound. However, the small transient increase in water content of unburned tissues of these rats was far less than that reported to occur in humans (7), a difference probably related to the fact that the burned rats did not receive intravenous fluid resuscitation.

Although we did not make albumin measurements as frequently during this early postburn period, it appears that albumin accumulation followed a course similar to that of water accumulation in the burn wound, since both reached maxima at about the same time after injury.

The phasic nature of water accumulation in rat burn wound (BU) paralleled changes in local blood flow which occur following burn injury (8). The water content of the burn wound (BU) increased rapidly during the period of increased blood flow, slowly during the period of relative stasis resulting from hemoconcentration, and again increased sharply as blood flow was re-established in the area which was not irreversibly damaged when the rats had become fully awake and begun to drink thirstily.

Because the plasma and burn wound albumin pool sizes of burned rats (BU) were approximately equal 1 hour postburn and the relative increases in albumin and water content were in the same proportions in which they are normally present in plasma, it appeared that for a time immediately after injury there was little or no restriction of transcapillary movement of water and albumin from plasma into the burn wound. Although we did not measure other proteins in the wound, it has been shown that essentially all sizes of plasma proteins move rapidly into the burn wound during this early postburn period (9,10).

The burn wound at 1 hour postburn contained the equivalent of approximately 1-1.5 ml plasma more than the plasma volume deficit observed at that time, indicating that some fluid had been translocated from other tissues before oral intake of water was begun. Although the extracellular water content of skin is greater than that of other tissues, there was no evidence that this was the source of the extra fluid. Tissues of liver and

7. Baxter CR: Fluid volume and electrolyte changes in the early postburn period. Clin Plast Surg 1:693-703, 1974.

8. Savitt S: Acute inflammatory changes in burned skin. In Burns Pathology and Therapeutic Applications, Butterworth & Co., London, 1957, pp 28-51.

9. Roberts JC, Courtice PC: Immunoelectrophoretic analysis of proteins in lymph from the leg before and after thermal injury. Australian J Exp Biol Med Sci 47:435-446, 1969.

10. Arturson G: Microvascular permeability to macromolecules in thermal injury. Acta Physiol Scand Suppl 463:111-122, 1979.

the gastrointestinal tract have been shown to respond most rapidly (11) to changes in plasma volume, so it is likely that the fluid was translocated into plasma from those tissues.

Labeled Albumin Studies

Conventional methods of analysis of labeled albumin disappearance data are based on the assumption that approximately 3 days after injection of the labeled albumin, the specific activities (SA) of albumin in the intravascular and extravascular albumin pools are equal. The size of the intravascular albumin pool is measured directly, and the size of the extravascular albumin pool is determined by relating the extravascular radioactivity to the plasma albumin SA. In addition, in studies of albumin metabolism in burned humans, catabolic rates have been determined by relating the plasma albumin SA at the mid-point of a measurement period to changes in extravascular radioactivity determined by whole body counting (12) or to urine/plasma ratios of radioactivity (13). Synthesis rates were estimated from the difference in the values for the catabolic rate and turnover rate determined from the slopes of the plasma albumin SA disappearance curves.

In normal humans or animals these methods yield fairly accurate estimates of pool sizes and turnover rates because muscle and skin, which constitute the major fraction of the body mass and contain the bulk of the extravascular albumin, have similar albumin exchange rates (14). Liver and tissue of the gastrointestinal tract have much more rapid exchange rates but contain only a small fraction of the extravascular albumin. As a consequence, the SA of albumin returning to plasma through the lymphatics is not greatly different from the average SA of albumin in the larger fraction of the extravascular albumin pool.

The situation proved to be quite different in the burned rats (BU). In burned rats injected with labeled albumin just before burn, wound and plasma SA and pool sizes were approximately equal at 1 hour postburn -- a time when very little labeled albumin was present in other nonvisceral tissues. In this case, the SA of albumin returning to plasma through the lymphatics was not representative of the average extravascular albumin SA but was instead a mixture of high SA albumin returning from burn wound and low SA albumin returning from other tissues. As a consequence, the

11. Walcott WW: Blood volume in experimental hemorrhagic shock. *Am J Physiol* 143:247-253, 1945.

12. Birke G, Liljedahl S-O, Plantin L-O, Reizenstein P: Studies on burns. IX. The distribution and losses through the wound of ^{131}I -albumin measured by whole body counting. *Acta Chir Scand* 134:27-36, 1968.

13. Davies JWL, Ricketts CR, Bull JP. Studies of plasma protein metabolism. Part I. Albumin in burned and injured patients. *Clin Sci* 23: 411-423, 1962.

14. Studer RK, Morgan J, Pankoske M, Potchen EJ: Regional vascular volume and extravascular accumulation of labeled protein during plasma volume expansion. *Am J Physiol* 224:699-704, 1973.

slopes of the labeled albumin disappearance curves of burned rats (BU) were not as smooth as those of sham rats (SU) whether plotted as albumin SA or as percent of dose remaining.

Because of the marked change of the proportions of albumin present in the intravascular and extravascular compartments of burned rats which occurs immediately after burn injury, and because the albumin pool sizes and hourly exchange rates continue to change with time, the outcome of a particular labeled albumin study would be greatly dependent upon the time of the injection of the labeled albumin. We found that in burned rats (BU) injected 24 hours postburn, when they appeared to have returned to a relative steady state (based upon stable albumin pool sizes and hourly transfer rates from 24-72 hours postburn), albumin SA in the burn wound was twice that of plasma albumin SA 5 days after the injection. Determining the wound albumin pool size from the total radioactivity and the plasma albumin SA, as has typically been done in human studies, would have yielded values twice those we obtained by direct measurements. Because we believe that the altered albumin exchange rates in the burned rats are a consequence of changes in the physical characteristics of the tissue of the burn wound, we do not believe that these findings are limited to burned rats, but are probably relevant to labeled albumin exchange studies in burned humans as well. It appears unlikely that one can accurately predict that extravascular albumin SA and intravascular albumin SA will be equal at any particular time in the presence of burn injury.

The steeper slope of the segment of the labeled albumin disappearance curves for the early postburn period of the hyaluronidase-treated burned rats (BHY) can be explained by the fact that the rate of albumin flow through the treated wound was much greater than that through the wound of untreated burned rats (BU) with the result that the wound albumin pool of rats in Group BHY never attained the size of the wound albumin pool in rats in Group BU. However, a comparable improvement in the plasma albumin pool size of rats in Group BHY was not observed. In fact, the plasma albumin pool of rats in Group BU returned to normal size more rapidly than that of Group BHY.

Although it is generally agreed that increased capillary permeability is the precipitating factor in edema formation following burn injury, recent studies show that interstitial tissue plays an important role in the control of fluid shifts between plasma and tissues (15). The connective tissue of the interstitium has been characterized as a two-phase system in which the mucopolysaccharides form a tight gel into which water can penetrate (16). This two-phase system is in osmotic equilibrium and

15. Guyton AC, Taylor AE, Granger HJ: Dynamics of edema and the safety factor against edema. *In* Circulatory Physiology. II. Dynamics and Control of the Body Fluids, A.D. Guyton et al, Eds., W.B. Saunders, Philadelphia, 1975, p 150.

16. Gersh I, Catchpole HR: The nature of ground substance of the connective tissue. *Perspect Biol Med* 3:282-319, 1960.

the partition of fluid between the two phases is dependent upon the relative concentrations of mucopolysaccharides and proteins in the tissue.

Guyton et al (17) have shown that interstitial pressure increases in proportion to the increase in fluid volume until the increase in pressure exceeds 8-10 mm Hg. Compliance then rises steeply and further increase in volume elicits very little increase in interstitial pressure, with the result that fluid moves freely into the interstitium. After this point, the upper limit of fluid volume increase is determined by the tensile elements of the tissue, primarily the skin. However, skin exhibits very little elastic recoil until it has been stretched to 1.5 to 2 times its normal length (15), so that large shifts of fluid from plasma can be expected to occur before such tension becomes a limiting factor. This state appears to be reached very rapidly following burn injury.

When the concentration of long chain hyaluronic acid molecules in the interstitium is reduced either by dilution (edema) or by depolymerization (action of hyaluronidase), the resistance to movement of water and proteins into and through the tissue is decreased. The fluid vesicles may coalesce to form pools of fluid through which molecules may diffuse freely. The prolonged elevation in the albumin and water content of the burn wound (BU) probably results from such changes in the physical characteristics of the interstitial tissue. The smaller increase in albumin and water content of the hyaluronidase-treated burn wound (BHY) reflects accelerated transport through an interstitial tissue in which the concentration of long chain hyaluronic acid molecules is further reduced by depolymerization. Although the albumin content of the hyaluronidase-treated burn wound (BHY) was lower than that of untreated burn wound (BU), a comparable improvement in the plasma albumin pool size of BHY was not observed. In fact, the plasma albumin pool of rats in BU was restored to normal more rapidly than that of BHY.

In summary, by determining the changes in water content and labeled and unlabeled albumin in wounds of rats injected with labeled albumin just before burn or sham burn, we have been able to show that the plasma and wound albumin pools of burned rats (BU) reach equilibrium within 1 hour after injury. Because the relative proportions of albumin in the plasma and wound albumin pools of BU remain essentially constant from 1-144 hours postburn, it appears that the two pools also reach dynamic equilibrium rapidly.

Although the SA of plasma and wound albumin in burned animals were equal 1 hour postburn, this equality soon disappeared. The plasma albumin SA curve of BU was similar to the plasma and wound albumin SA curves of SU after 48 hours (as it is assumed to be in the customary models used for determining albumin turnover). However, even though the hourly exchange

17. Guyton AC, Taylor AE, Granger HJ: Pressure-volume curves of the interstitial fluid spaces. *In* *Circulatory Physiology. II. Dynamics and Control of the Body Fluids*, A.C. Guyton et al, Eds., W.B. Saunders, Philadelphia, 1975, pp 71-86.

rates of albumin in the wounds of the burned rats were greater than those of the controls, their wound albumin SA curves declined more slowly. Because of the presumed changes in the physical properties of the connective tissue of the interstitium, in the size of the wound albumin pool, and in the exchange rates of labeled albumin, the burn wound, in effect, becomes an additional extravascular compartment exhibiting dynamics which cannot be satisfactorily described by the slopes of the plasma albumin disappearance curves or by the whole body counting methods which have been used in the past.

Hyaluronidase treatment of the burn wound resulted in an acceleration of the rate of albumin exchange between plasma and wound during the early postburn period but the wound albumin SA curve from 48-144 hours in such animals was similar to that of untreated burned rats, and plasma albumin pool size was more slowly restored.

PRESENTATIONS/PUBLICATIONS - None.

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

**REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS
OF BURN INJURY IN SOLDIERS -- A NEW APPROACH TO
THE STUDY OF THE HYPERMETABOLIC RESPONSE TO
THERMAL INJURY**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**L. Howard Aulick, Ph.D., Lieutenant Colonel, MSC
Hartmut Arnhold, A.E.
Edwin W. Hander, M.A.
Arthur D. Mason, Jr., M.D.**

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

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US Army Institute of Surgical Research, Brooke Army Medical Center
Fort Sam Houston, Texas 78234

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Investigators: L. Howard Aulick, Ph.D., Lieutenant Colonel, MSC
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The open and closed respiration chamber, described in the previous annual report (1) has been constructed and is currently being automated and calibrated. This report will review the basic design features and describe the operation of the chamber as well as discuss the initial calibration data.

Metabolic response
Thermoregulatory response

THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN
INJURY IN SOLDIERS -- A NEW APPROACH TO THE STUDY OF
THE HYPERMETABOLIC RESPONSE TO THERMAL INJURY

Chamber Design and Operation. The chamber is a 8x4x4-foot box made of 3/16-inch steel and covered with a one-inch layer of thermal insulation (Figure 1). Two spirometers are attached - a 10-liter, low resistance spirometer (Model 840, Ohio Medical Products, Madison, Wisconsin) and a 120-liter spirometer (W.E. Collins, Inc, Braintree, Massachusetts). A small fan continuously moves air from the chamber through the large spirometer. The chamber has a large door of 1/2-inch plexiglas and a small observation window of the same material. Both are covered with an opaque screen to reduce heat exchange and permit undetected observation of the experimental animal.

The animal space is large enough to accommodate individuals or animals up to 150 kilograms. The floor is made of a heavy, expanded metal screen which is elevated 1 inch above the chamber floor. The animal is free to move about in this area but does not have access to the air treatment corridor, an 8x1x4-foot channel running the length of the back wall. Chamber air is continuously circulated by four fans in the air treatment corridor. The air travels at a velocity of 200 feet per minute and moves freely to and from the animal space (as indicated by the large arrows) through screens at either end of the air treatment corridor. Fluorescent lights behind these screens illuminate the animal space. When the chamber is closed, air moves from the fans through shutters and past a set of cold coils where water vapor is removed. A compressor behind the chamber keeps the ethylene glycol entering the cold coils between 0-5°C. Cool, dry air then passes through hot coils where it is returned to the desired temperature. Chamber temperature is controlled by varying the temperature of water in the hot coils. Water temperature is regulated by a proportional controller (Model 72, Yellow Springs Instruments, Yellow Springs, Ohio) which senses air temperature in the animal space and varies the output

1. Aulick LH, Arnhold H, Hander EW, Rodkey WG, Mason AD, Jr.: A new approach to the study of the hypermetabolic response to thermal injury. USAISR Annual Progress Report

of a 1000 watt immersion heater in the water reservoir. Using this system, average air temperature can be maintained as low as 2°C to as high as 40°C. When the ports on the roof are opened, shutters close and chamber air leaves by the exhaust port to be replaced by room air entering through the intake port. Under normal operating conditions, it usually takes about 3-5 minutes before chamber O₂ and CO₂ concentrations equal those in the laboratory.

Port covers are shallow cylinders, 10" in diameter and made of 3/16" steel. They are raised and lowered by a small electric motor. In the closed position, the rim of each port rests on the roof of the chamber in a circular trough. This trough contains light-weight mineral oil to prevent gas exchange across the closed port.

When the chamber is closed, it is hermetically sealed. This was demonstrated both by its capacity to maintain non-room air gas concentrations and a limited vacuum (5-10 mm H₂O) for extended periods of time. Chamber temperature is monitored by five, two-terminal, monolithic, integrated circuit, temperature transducers (Model AD590, Analog Devices, Norwood, Massachusetts). Two of these are located at either end of the air treatment corridor, while the fifth measures wall temperature of the animal space. Once air and wall temperatures equilibrate, they do not vary more than ± 0.2°C over a 24-hour period of operation. Chamber air temperature is considered the average of these five temperatures.

While chamber temperature is held constant, pressure varies with barometric. This is accomplished by movements of the low resistance spirometer. The pressure gradient across the chamber wall remains less than 0.5 mm H₂O. When changes in chamber gas volumes exceed the limits of this small spirometer, they are accommodated by compensatory adjustments in the position of the large motorized spirometer.

Measurement of Respiratory Gas Exchange. Respiratory gas exchange is determined by measuring the rate of change in oxygen and carbon dioxide volumes while the animal is confined in the closed chamber. Gas concentrations are measured separately in the chamber and large spirometer by a mass spectrometer (MGA Model 1100, Perkin-Elmer, Pomona, California). Oxygen (VO₂) and carbon dioxide (VCO₂) volumes are then determined by multiplying the gas fraction (FO₂ or FCO₂) times total volume (TV), where TV is the sum of the chamber (CV), small spirometer (SSV) and large spirometer (LSV) volumes minus animal volume (AV). All gas volumes are corrected to standard conditions. Air temperature in the chamber and small spirometer (CT) is considered the mean of the five temperature transducers whose locations were described earlier. Temperature of the large spirometer (ST)

is monitored separately. Pressure in the entire system (PBar) is measured by an electronic barometer (B242 Analog output barometer, Weather Measure Corp., Sacramento, California). Water vapor pressure is recorded in both the chamber (CPH₂O) and large spirometer (SPH₂O) by the mass spectrometer. The initial VO₂ would be calculated:

$$VO_{2stpd} = CFO_2 * \left[(CV + SSV - AV) * \left(\frac{PBar - CPH_2O}{760} * \frac{273}{273 + CT} \right) \right] + SFO_2 * \left[LSV * \left(\frac{PBar - SPH_2O}{760} * \frac{273}{273 + ST} \right) \right]$$

where CFO₂ and SFO₂ are chamber and large spirometer O₂ fractions respectively. These measurements and calculations are repeated when chamber CO₂ concentration reaches approximately 0.9 percent and the animal's oxygen consumption and carbon dioxide production considered the rate of change of these respiratory gas volumes over the period of confinement.

SSV and LSV can be very accurately determined from changes in the position of each spirometer. AV can be estimated from animal density and body weight. CV, however, must be very carefully determined, because it represents the largest single volume. For this reason, CV was estimated by three independent methods. The first was by physical measurement. Using this approach, the best estimate of CV was 3630 liters. The second technique was to introduce known quantities of pure argon gas into the chamber and calculate TV from the change in argon concentration. Eleven such dilution studies gave a TV of 3718 ± 4 liters (mean ± SE). Subtracting LSV and SSV from the calculated TV yielded a CV of 3653 liters, only 0.6 percent above that estimated from physical measurements. The third approach was to burn methyl alcohol at known rates in the chamber and calculate the volume of gas necessary (TV) to give the measured rates of O₂ disappearance and CO₂ accumulation (assuming full combustion and using predicted rates of O₂ consumption and CO₂ production). Once again, after deducting for LSV and SSV, the calculated CV was 3647 ± 20 liters (n = 39) when O₂ was the tracer gas and 3629 ± 18 liters when CO₂ was used. Since the four separate estimates of CV were in close agreement, an average value of 3640 liters was chosen. The greatest possible error in this estimate is 13 liters, or only 0.4 percent of the average CV.

Chamber Calibration. The accuracy of respiratory gas exchange measurements by this new system was tested by comparing observed rates of oxygen consumption (V_{O_2}) and carbon dioxide production (V_{CO_2}) with those predicted from known rates of methanol combustion. A total of 46 such studies were performed at three chamber temperatures (Table 1). In seven studies, no methanol was burned. Combustion rate was varied to provide a range of oxygen uptake from 4.250 to 23.376 liters/h. Each run began as soon as the chamber was closed and continued until CO_2 concentration reached 0.9 percent. Run duration ranged from $1\frac{1}{2}$ to 10 hours. (The zero runs lasted two hours.)

The error in the measured V_{O_2} was significantly greater in the $40^\circ C$ environment than in the other two environments but only represented an average overestimation of less than two percent of the predicted value (Table 1). A multiple regression, however, indicated that predicted V_{O_2} was the only significant determinant of measured V_{O_2} when the data from all three environments were included (Figure 2). In this case, over 99 percent of all the variation in measured V_{O_2} could be explained by the variations in rate of methanol combustion.

There was no significant difference in the mean CO_2 error among the three environmental groups, but the trend was to progressively overestimate V_{CO_2} as chamber temperature was reduced (Table 1). A multiple regression revealed that a very small component of the variation in measured V_{CO_2} could be explained by these differences in environmental temperature. But, since the temperature effect only increased the index of determination (r^2) from 0.9995 to 0.9996, it was considered functionally insignificant (Figure 3).

The measured RQ ranged from 0.64 to 0.72 and on the average was lower in the heat than in the other two environments (Table 1). This was a result of the previously described overestimation in measured V_{O_2} at this temperature. Since the predicted RQ for methanol is 0.667, the measured mean of 0.658 in the heat represents a 1.3 percent error. When all the studies are included, the measured RQ was not significantly affected by the rate of alcohol combustion (as indicated by measured V_{O_2}) and averaged 0.671, or within 0.6 percent of the predicted volume (Figure 4). Twenty-six of the 39 measures were within ± 0.01 of the predicted value.

The results of these calibration studies indicate this new open and closed respiration chamber measures respiratory gas exchange with great accuracy. These tests, however, were performed manually prior to implementing computer automation of chamber operation and data acquisition. This automation procedure is currently underway, and the process of calibration will be repeated before animal studies begin.

TABLE 1
METHANOL COMBUSTION (MEAN \pm SE)

<u>NUMBER OF STUDIES</u>	15	16	15
<u>CHAMBER TEMP.</u> (°C)	40.2 \pm 0.2	25.4 \pm 0.1	10.6 \pm 0.1
<u>OXYGEN CONSUMPTION</u> (LITERS/HOUR)			
1. PREDICTED	11.857 \pm 2.026	10.778 \pm 1.758	11.274 \pm 1.871
2. MEASURED	12.086 \pm 2.100	10.809 \pm 1.788	11.303 \pm 1.859
3. ERROR*	0.228 \pm 0.121	0.031 \pm 0.085	0.028 \pm 0.069
<u>CARBON DIOXIDE PRODUCTION</u> (LITERS/HOUR)			
1. PREDICTED	7.928 \pm 1.355	7.206 \pm 1.175	7.538 \pm 1.251
2. MEASURED	7.950 \pm 1.396	7.268 \pm 1.200	7.694 \pm 1.288
3. ERROR*	0.023 \pm 0.053	0.063 \pm 0.030	0.156 \pm 0.042
<u>RESPIRATORY QUOTIENT</u>	0.658 \pm 0.005	0.678 \pm 0.004	0.678 \pm 0.005

*MEASURED-PREDICTED

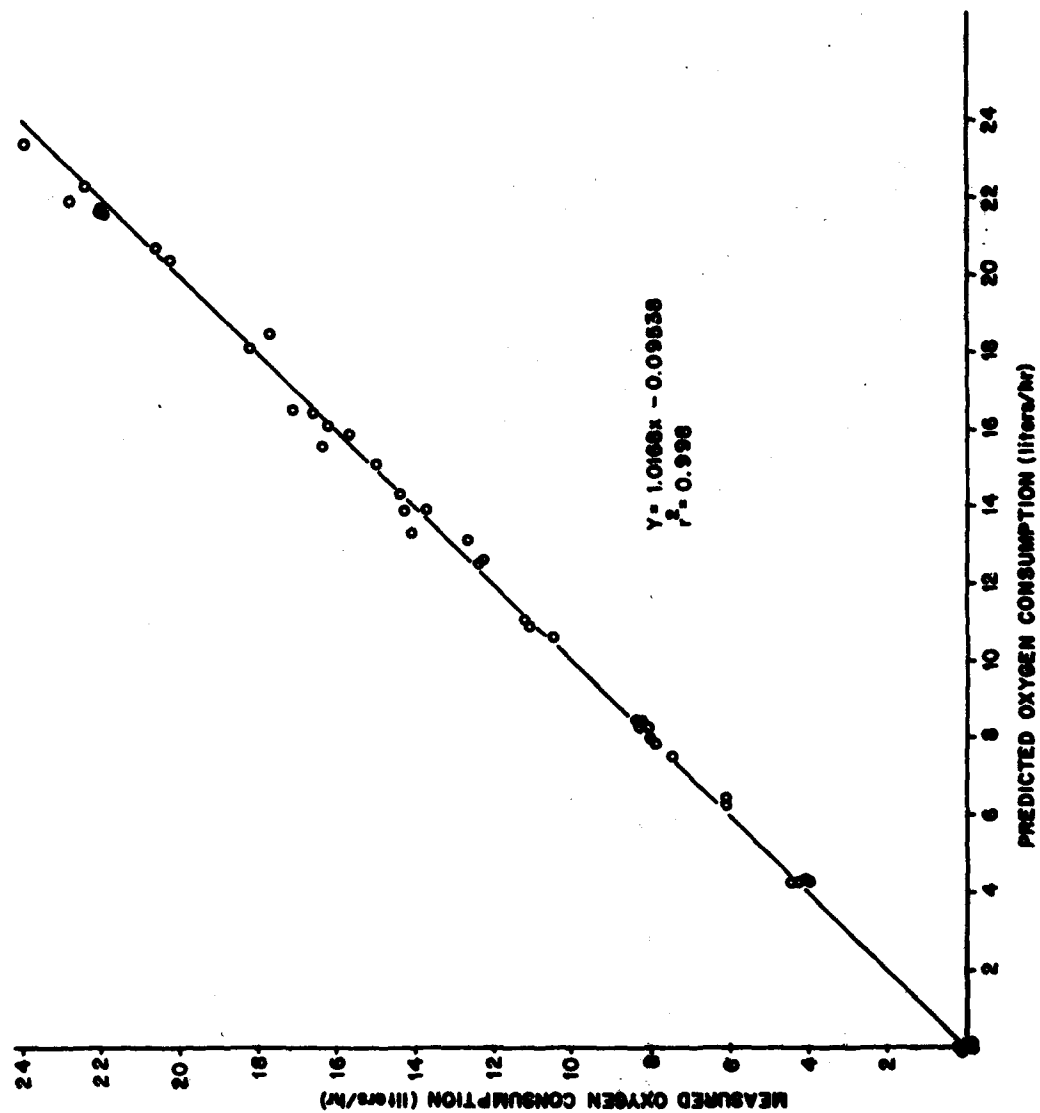


FIGURE 2.

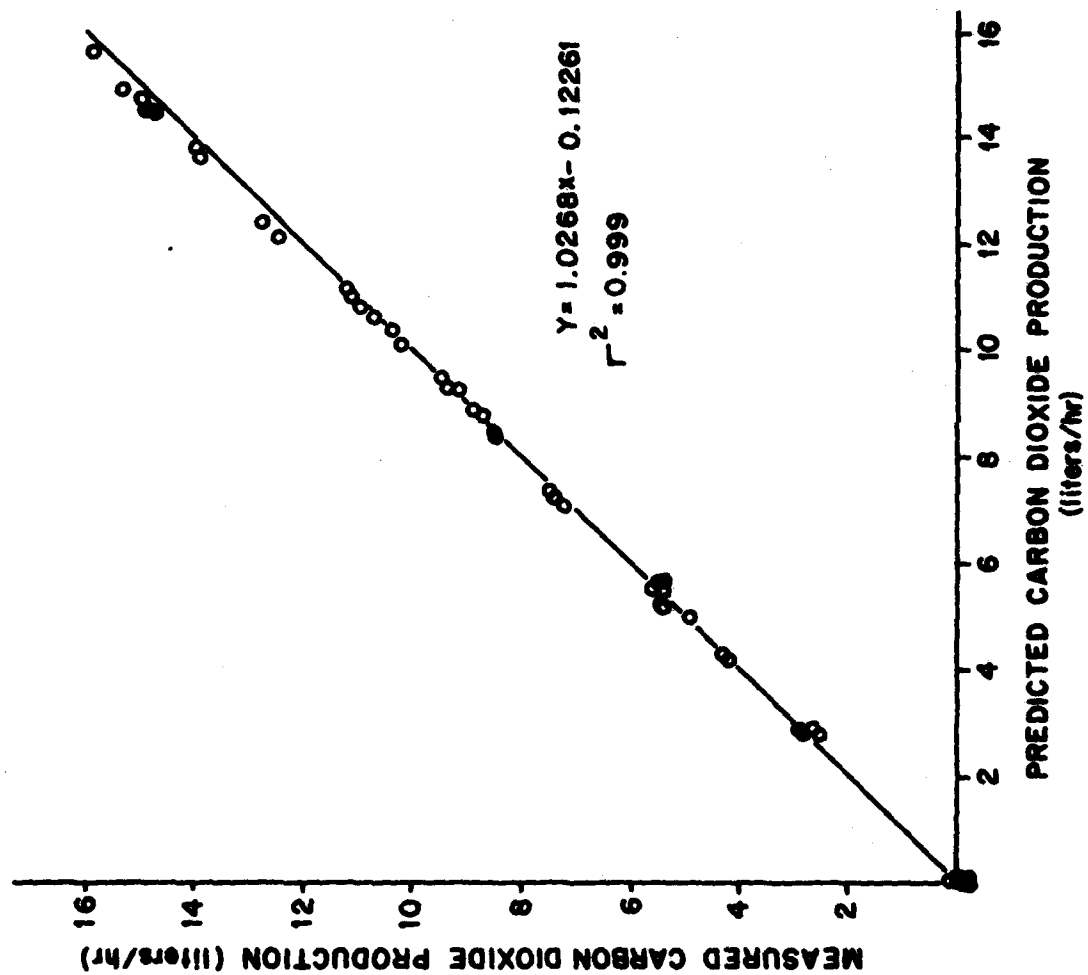


FIGURE 3.

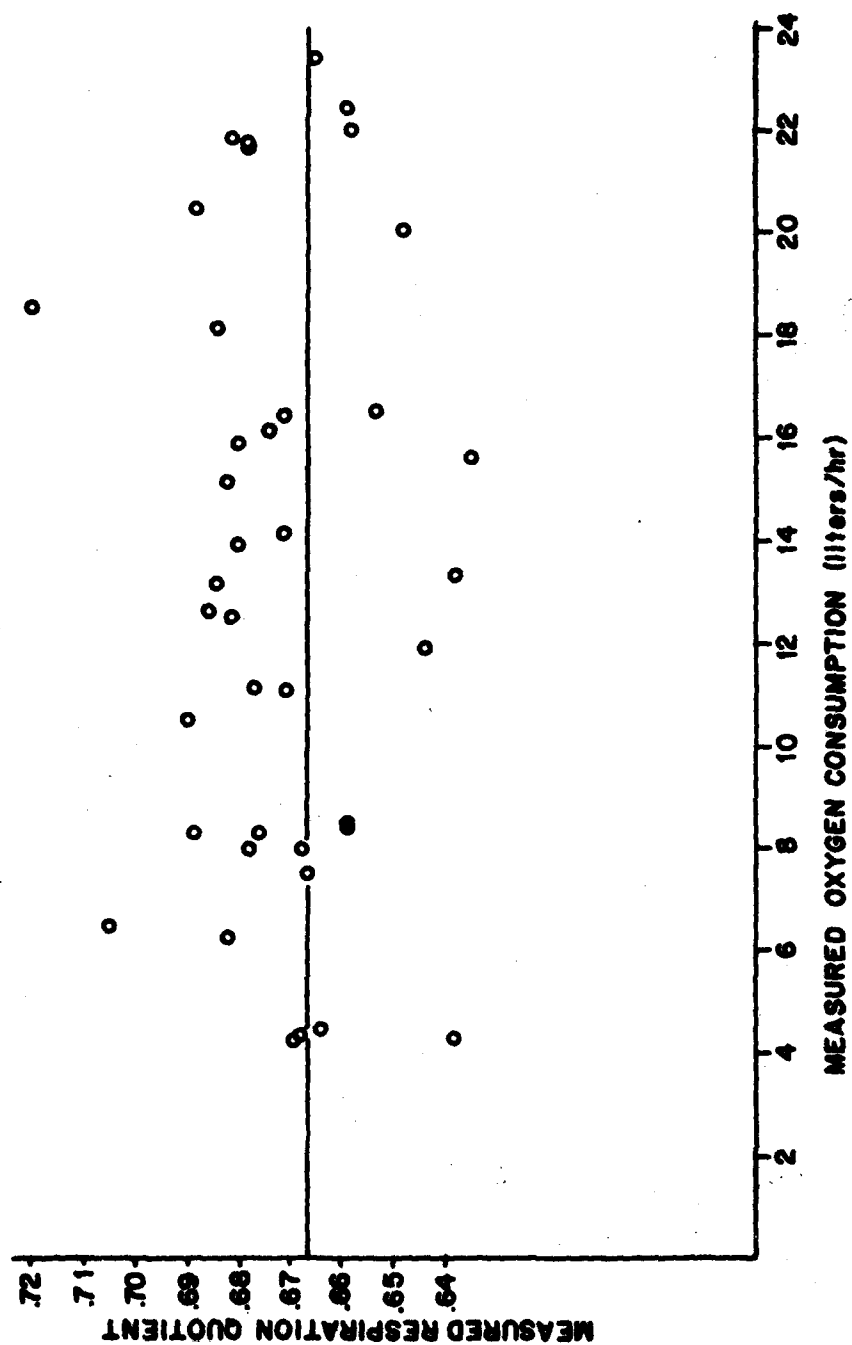


FIGURE 4.

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

**REPORT TITLE: COMPUTER GENERATED GRAPHIC EVALUATION OF
NUTRITIONAL STATUS IN CRITICALLY INJURED PATIENTS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**Nancy K. McLaurin, R.D., Captain, AMSC
Cleon W. Goodwin, Jr., M.D.
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Reports Control Symbol MEDDH-288 (R1)

Unclassified

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Reports Control Symbol MEDDH-288(R1)

A continuous computer graphics program was developed to aid evaluation of the nutritional state of critically ill burn patients quickly and efficiently without having to read through and calculate long lists of chart entries.

Initial assessment of metabolic expenditure and nutritional requirements of severely burned patients is made using algorithms stored in a computer program. The computer algorithms for predicting metabolic requirements correlate closely with physiologic data obtained by direct measurement ($R=0.84$ for metabolic resting energy expenditure). Each patient's diet (parenteral and/or enteral) is tailored to meet these requirements. The enteral diet is prepared individually in the Institute's metabolic kitchen. The parenteral diet is prepared by the hospital pharmacy following the physician's prescription, which is based on the nutritional support team's recommendations. The nitrogen and calorie intake are recorded daily and the ratio is maintained between 1:120 to 1:180. A weight loss exceeding ten percent of the preinjury weight dictates reevaluation of the patient's metabolic expenditure by indirect calorimetry and reformulation of his/her nutrition support plan. The computer generates daily profiles of predicted calorie and protein requirement, actual intake with percentages of requirement, nitrogen/calorie ratio, weight changes, and nitrogen balance (nitrogen loss is calculated from twenty-four hour urine urea excretion with adjustments for stool and wound losses). A continuous computerized graphics display summarizes all the nutritional data for each patient by plotting weight, calorie and protein balances.

Critically ill
Metabolic expenditure
Continuous computer graphics

COMPUTER GENERATED GRAPHIC EVALUATION OF NUTRITIONAL STATUS IN CRITICALLY INJURED PATIENTS

NUTRITION AND THE CRITICALLY ILL

The metabolic response to trauma is initiated in large part by the central nervous system and is manifested by the release of antidiuretic hormone (ADH), adrenocorticotrophic hormone (ACTH), catecholamines and glucagon (1-4). These hormonal changes appear to be essential for the maintenance of homeostasis and survival (5). Following trauma, protein and fat catabolism increase (2) to supply energy requirements, water and sodium are retained (3), and potassium excretion is increased (5). The intensity of the response is in proportion to the degree of the injury (3,4,6) but will vary with age, sex and previous nutritional status (7).

The effects of this hormonal response may continue for several weeks. In such cases, prolonged muscle proteolysis and lipolysis may result in muscle weakness, respiratory failure, sepsis, and eventually death if the sequelae of posttraumatic hypercatabolism are not reversed or eliminated. The provision of adequate nutrition is of vital importance in avoiding the depletion of body tissue and in initiating the anabolic repair phase (5). Without exogenous nutrition, the body's limited stores of carbohydrate may be depleted in eight to ten hours (5) after trauma.

-
1. Blackburn GL, Bistrian BR: Nutritional care of the injured and/or septic patient. *Surg Clin North Am* 56(5): 1195-1224, 1976.
 2. Elwyn DH, Kinney JM, Jeevanandam M, Gump FE, Broell JR: Influence of increasing carbohydrate intake on glucose kinetics in injured patients. *Ann Surg* 190(1): 117-127, 1979.
 3. Wilmore DW: Hormonal responses and their effects on metabolism. *Surg Clin North Am* 56(5): 999-1011, 1976.
 4. Kauste, A.: Parenteral and enteral nutrition of the thermally injured patient. *Ann Chir Gynaecol* 69: 197-201, 1980.
 5. The Doyle Pharmaceutical Company: *Nutrition in Trauma and Stress - Reference Manual*. A Division of the Delmark Company, Inc., 1980.
 6. Wolfson, AMJ, Heatley R, Allison SP: Insulin to inhibit protein catabolism after injury. *N Engl J Med* 300(1): 14-17, 1979.
 7. Randall HT: Nutrition in surgical patients. *Am J Surg* 119: 530-533, 1970.

At this time, energy demands must be met by amino acids and fatty acids supplied by the breakdown of body proteins and fats (7). Twenty-five to thirty percent weight loss has been associated with high risk of mortality (6,8) in critically ill patients. Nitrogen loss may go as high as 15 to 25 gm per day in patients with such severe injuries as burns or multiple fractures with sepsis. In the early stages, the effects of stress can be minimized but not corrected by adequate nutritional support (1,7,9). However, preservation of body cell mass and survival cannot be attained unless nutritional support is adequately provided (5).

EVALUATION OF NUTRITIONAL STATUS USING COMPUTER PROGRAM

The Institute of Surgical Research (ISR) nutritional support system consists of a series of computer programs and data files which allow the computation of nutritional requirements for critically injured patients and an evaluation of the adequacy of nutritional therapy in meeting these requirements.

The system runs on a Digital Equipment Corporation model PDP-11/70 computer system with 512K bytes of main memory, using the RSX-11M+ operating system. All programs run in an interactive manner on VT-100 video terminals, using video forms for both input and display of information.

When desired, summary graphs of stored information can be produced showing weight balance, caloric balance, and protein balance. Printed daily, summaries are produced for inclusion in patient records and provide a complete listing of nutrients received, the route of administration, and the percentage of the predicted requirements for each individual patient.

INITIAL DIETARY ASSESSMENT OF NUTRITIONAL REQUIREMENTS

Calories: Caloric needs can be determined by direct or indirect calorimetry with a high degree of accuracy. However, these techniques are expensive, time consuming and usually not available. Therefore, numerous formulas for estimating the required caloric intake for critically ill patients have been developed. (For specific formulas, refer to references

8. Blackburn GL, Flatt JP, Clowes GHA Jr, O'Donnell TF, Hensle TE: Protein sparing therapy during periods of starvation with sepsis or trauma. *Ann Surg* 177(5): 588-593, 1973.

9. Cuthbertson DP: Further observations on the disturbance of metabolism caused by injury. *Br J Surg* 23(91): 505-520, 1936.

10-14). Formulas for predicting nutritional requirements (Fig. 1) in thermally injured patients at the Institute were derived from measurements of metabolic expenditure by indirect calorimetry.

Protein: The nitrogen-calorie ratio in normal adults during nitrogen equilibrium is approximately one gram of nitrogen for every 350 calories. Due to the decrease in protein economy that occurs with most critical illness, a one to 150 nitrogen-calorie ratio appears to effect nitrogen balance in critically ill patients (1,14,15) although the ratio may range between 1:100 to 1:200.

Vitamins: Major injury or stress increases the requirements for vitamin C, niacin, thiamine and riboflavin. Vitamin requirements usually are met by a balanced diet, but oral supplements may be administered when necessary (14).

Minerals: Provision of minerals is essential to the nutritional and physiologic support of the critically ill patients. Minerals usually are adequately provided in most oral diets or prepared feeding formulas. The adequacy of intake of the major mineral substances may be determined by frequent monitoring of serum levels or assay of the biological fluids or tissues and supplemented as needed (14).

ROUTINE DIETARY ASSESSMENT OF INTAKE

The priorities of the care of injured patients mandate that factors such as fluid resuscitation, fluid and electrolyte balance, and optimal cardiovascular function are more important than achieving a nutritional balance during the early postinjury period (14,16,17). Nutritional replacement should start as

10. Curreri PW, Richmond D, Marvin J, Baxter CR: Dietary requirements of patients with major burns. *J Am Diet Assoc* 65: 415-417, 1974.

11. Sutherland AB: The nutritional care of the burned patient. *Br J Plast Surg* 8: 68-74, 1955.

12. Troell L, Wrethling A: Protein and calorie requirements in burns. *Acta Chir Scand* 122: 15-20, 1961.

13. Artz CP, Soroff HS, Pearson E, Hummel RP: Some recent developments in oral feedings for optimal nutrition in burns. *Am J Clin Nutr* 4: 642-646, 1956.

14. Wilmore DW: *The Metabolic Management of the Critically Ill*. 2nd ed. New York: Plenum Medical Book Co., 1980.

15. Larkin JM, Moylan JA: Complete enteral support of thermally injured patients. *Amer J Surg* 131: 722-724, 1976.

16. Artz CP, Moncrief JA, Pruitt BA: *Burns: A Team Approach*. Philadelphia: WB Saunders Company, 1979.

17. Curreri PW: Nutritional replacement modalities. *J Trauma* 19: 906-908, 1979.

soon as this initial phase of treatment is completed. Three routes of administration may be employed: gastrointestinal tract, peripheral vein, or central vein. When treating burn patients, special emphasis should be given to patients with greater than 20 percent total body surface burn, to patients who have preinjury nutritional deficiencies (including prior illness, morbid obesity, alcohol or drug abuse, and child neglect), to burned patients with associated injuries, to any patient with a more than ten percent preburn weight loss, and to patients with severe endocrinologic, pulmonary, or septic complications (17). At the Institute, routine dietary assessment of intake is done for any patient who fits into one or more of the above categories. This assessment is done using a computerized program (Fig. 2) which provides the user with the following information: total intake of calories, protein, fat, carbohydrate, most minerals and most vitamins; route of administration; percentage of predicted requirement consumed (Fig. 1); nitrogen to calorie ratio, nitrogen balance; and percentage of weight change.

GRAPHIC REPRESENTATIONS

Summary graphs of previously stored information (Fig. 1) can be produced showing weight balance, caloric balance and protein balance (Figs. 3,4). The individual patient's requirements as well as his preburn weight are displayed on the graphic display, enabling the staff, patient, and family members to readily observe the patient's current nutritional status in relation to his estimated requirements. The shaded area on the weight balance graph indicates a weight loss range of ten percent or more from the preburn weight. The graphs are printed in easy to understand language.

Once the graphs are made, they are displayed at the bedside of each of the patients, showing him and his family his progress or lack of it. With the aid of the dietitian, the patient can learn how to progress more quickly and how to turn a negative balance into a positive one.

For the nursing staff, which often does not have time to go through long lists of chart entries to determine each patient's nutritional balance, the graphs are a useful tool. Patients are more easily persuaded to increase their dietary intake. Also, the nursing staff becomes more aware of empty calorie supplements such as juices and instead can offer milk shakes and other supplements between meals.

For other members of the health team, the graphs serve as a constant reminder of the importance of nutritional support in the recovery of the critically ill patients. This technique also indicates when the route of administration needs to be reassessed, and/or supplemented in order to meet the requirements.

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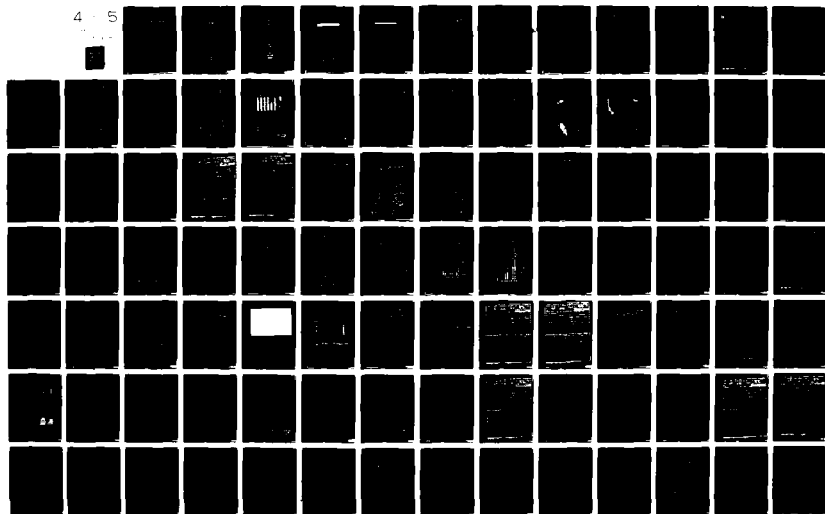
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CONCLUSION

The pictorial display of the patients' daily nutritional status not only alerts the medical care team to potential deficiencies but also facilitates utilization of caloric and protein supplements to meet nutritional goals. Further, the family members and patients often are encouraged by this information to gear their efforts toward these goals. The display of the graphs at the bedside serves as a constant reminder of the importance of nutritional support in the recovery of the critically ill patient.

ISR ASSESSMENT AND SUMMARY PROGRAM

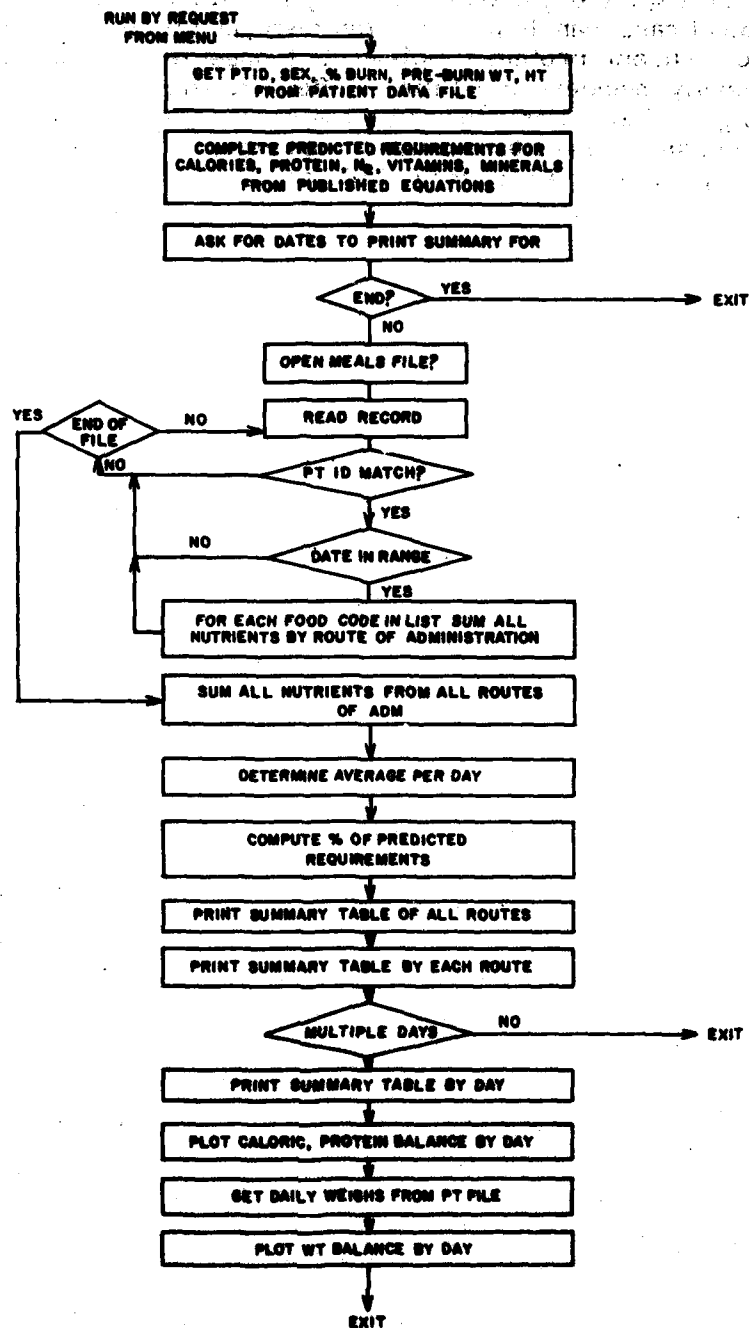


Figure 1

ISR CALORIE COUNT PROGRAM FLOW CHART

PROGRAM CALLED BY MENU REQUEST

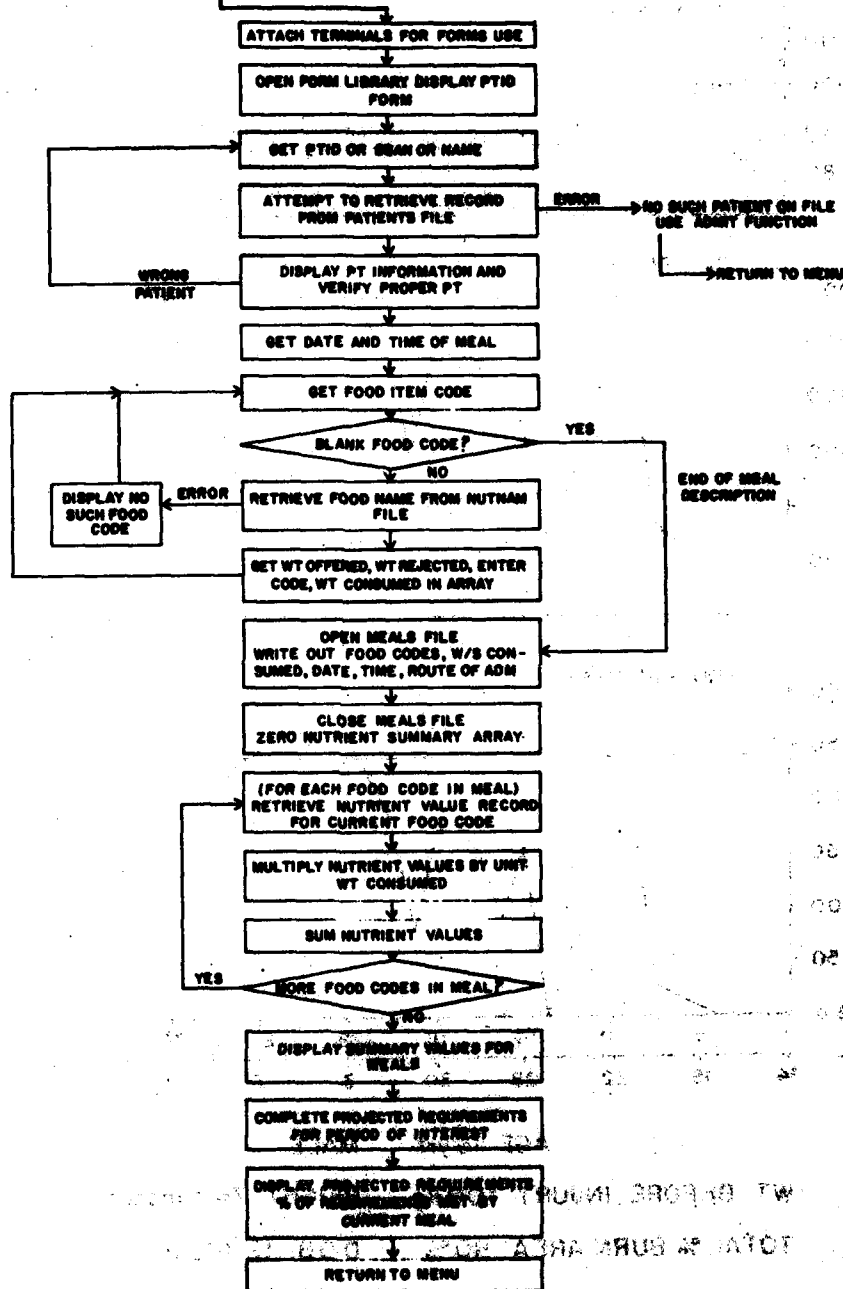
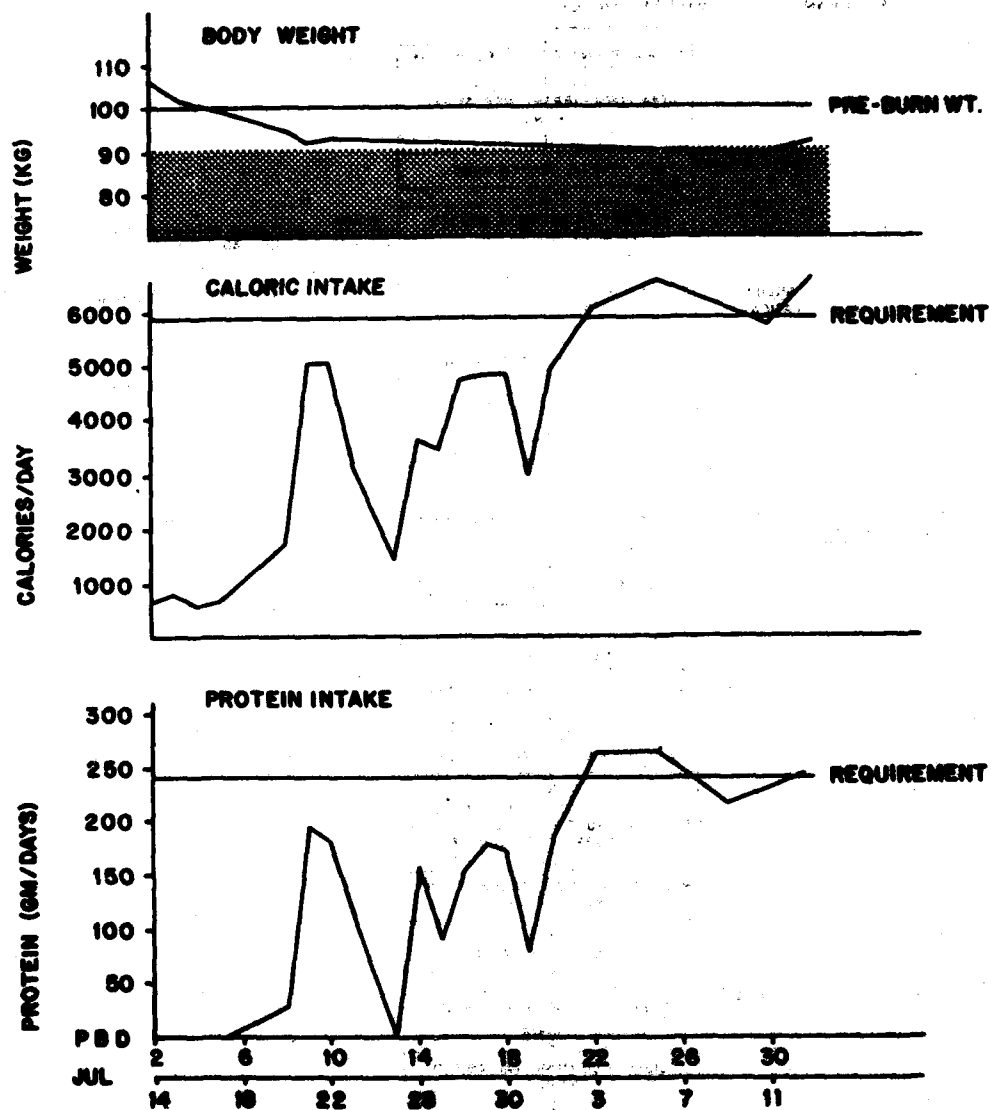
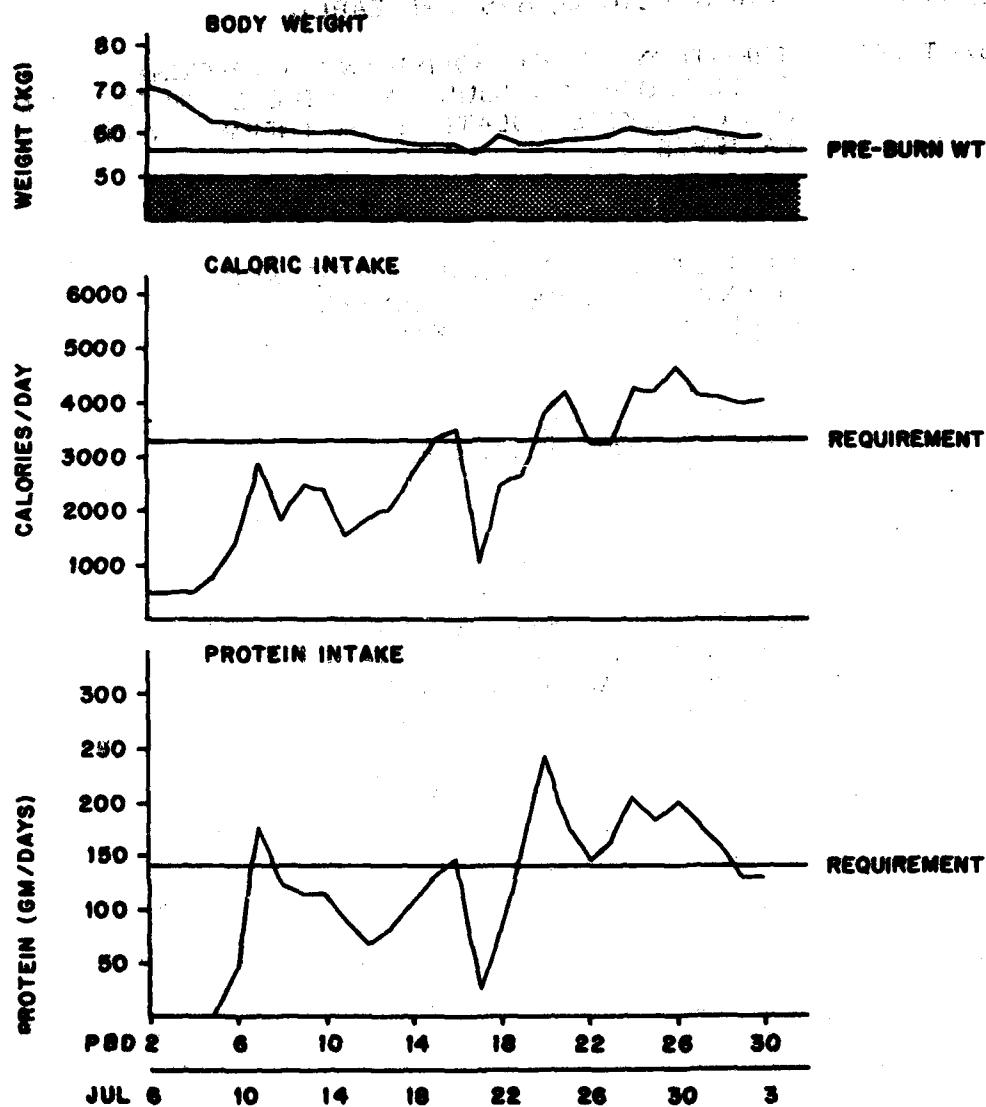


Figure 2



AGE: 16 yrs. MALE
 WT. BEFORE INJURY: 100 kg. HEIGHT: 74.5 inches
 TOTAL % BURN AREA: 80% DOB: 12 JUL 61

Figure 3



AGE: 35 yrs. MALE

WT. BEFORE INJURY: 56.2kg HEIGHT: 62 inches

TOTAL % BURN AREA: 60% DOB: 4 JUL 81

Figure 4

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

**PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL
EFFECTS OF BURN INJURY IN SOLDIERS - METABOLISM
OF ADIPOCYTES ISOLATED FROM THERMALLY INJURED
PATIENTS**

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Investigators:

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Reports Control Symbol MEDDH-288(R1)

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Reports Control Symbol MEDDH-288(R1)

One characteristic of the hypermetabolic period following severe thermal injury in humans is an elevated mobilization of body lipids. The isolated adipocyte has been chosen as a controlled environment for determining the function of adipose tissue in normal and injured systems. Preliminary experiments have been completed to confirm the effectiveness of this method in observing lipolytic rates in our laboratory. Initial values for rates of triglyceride breakdown and responsiveness to hormonal stimulation are comparable to values obtained from an animal model.

Hypermetabolic
Adipocyte
Lipolysis
Triglyceride

THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS - METABOLISM OF ADIPOCYTES ISOLATED FROM THERMALLY INJURED PATIENTS

Elevated plasma and urinary catecholamines, increased glucose flow and accelerated triglyceride turnover characterize the hypermetabolic state observed in thermally injured patients (1). During this period, there is an increased use of fatty acids derived from adipose tissue, as suggested by increased catecholamines (lipolytic agonist) (2, 3), elevated glycerol turnover (4), fat loss (5, 6), fatty acid deficiencies (7) and elevated plasma fatty acids (8). Although there is a large increase in lipid mobilization, its role in the overall metabolic scheme is not well defined. Provision of dietary fat is not useful in decreasing the loss of body nitrogen (9, 10), and the net oxidation of fat cannot be offset by excess glucose (11, 12).

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The isolated adipocyte demonstrates lipolytic hormonal response similar to intact tissue (12) and provides a controlled environment for determining the function of adipose tissue in normal and injured systems. Because of the major control exerted on lipolysis by catecholamines and the chronic elevation of these hormones in the burned patient, it was undertaken to determine the effect of hormonal stimulation on isolated adipocytes from humans. The experiments to date have been preliminary and designed to expose differences in animal and human tissue which occur in the technical procedures of tissue collection, cell isolation, incubation and fixing.

PATIENTS AND METHODS

The patient protocol has recently been initiated. The one patient study was a male with an 80% total body surface burn. Tissue which has obtained from this patient was placed in warm Krebs-Ringer phosphate buffer (KRP) and transported immediately to the laboratory. The tissue was minced and placed in buffer containing 4% by weight albumin fraction V (Sigma, Lot 80F07071) and 3 mg/ml collagenase (Worthington, Lot 40K043). After digesting at 37°C for one hour, the cells were separated from the tissue matrix by filtering through a 105 μ nylon mesh. These cells were washed three times with KRP and suspended in KRP-albumin. Duplicate 5 ml aliquots of this suspension were incubated in the presence and absence of 10^{-5} M epinephrine at 37°C for one hour. At the end of the incubation period, 4 ml of the suspension was pipetted into 0.4 ml cold trichloroacetic acid (TCA; 50% w/v). A separate pair of samples was added to TCA immediately upon dispensing to provide pre-incubation values. The TCA samples were filtered and the filtrates stored at -20°C until they could be analyzed for glycerol content. Glycerol production was determined as the difference in glycerol content between the one-hour incubations and those at time zero. This value was expressed as nmoles/ml-hr and served as an index of triglyceride breakdown. Final values were standardized per 10^6 cells by counting aliquots of cells from the original suspension which had been fixed in 2% osmium tetroxide (Degussa Corp.). The fixed cells were also measured microscopically for diameter, using an eyepiece reticle. Finally, an aliquot of the original suspension was dispensed into 20 volumes of 2:1 chloroform/methanol for extraction of triglycerides. Triglyceride content was determined by enzymatic assay (Sigma Kit 405).

Preparation of the adipocytes for all phases of the experiment was not found to be essentially different from tissue taken from rats. The main difference is in the amount of collagenous material in the human tissue. Mincing was much more difficult, and the residue after filtering through the nylon mesh was significantly greater than with rat epididymal tissue. This, however, did not affect the cell isolation in any discernible manner.

RESULTS

Values which were determined for the human tissue are listed below with representative values from experiments done in our laboratory on small animals (rats, 450-500 grams, 60% burn).

	<u>PATIENT</u>	<u>ANIMAL</u>
Basal Glycerol Production (nmoles/ 10^6 cells/h)	206.8	138
Stimulated Rate	971.9	1481
Difference due to Stimulation	765.1	1343
Cell Diameter (U)	81.3	70
Triglyceride (mg/ 10^6 cells)	450.3	170

At this time, it is not possible to say more than that the isolation technique results in cells which are viable in terms of lipolytic response to hormonal stimulation. The values obtained are reasonable in light of those obtained from animal studies. Although there appears to be a large difference in cell size and triglyceride content, more experiments will have to be done to see if this is a consistent finding.

PUBLICATIONS/PRESENTATIONS

None.

ANNUAL PROGRESS REPORT

PROJECT NO. 3M161102BS10-00, BASIC RESEARCH

**REPORT TITLE: ASSESSMENT OF L-TRIIODOTHYRONINE THERAPY IN
THERMALLY INJURED PATIENTS -- THE
HYPERMETABOLIC LOW TRIIODOTHYRONINE SYNDROME
IN BURNED SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1979 - 30 September 1980

Investigators:

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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Severely burned soldiers had suppressed indices of free thyroid hormone levels (FT_4 and FT_3) and normal levels of TSH without an augmented TSH response to TRH. FT_4 and FT_3 were more markedly depressed in those who subsequently died, and these patients had low TSH levels and a blunted and delayed TSH response to TRH. Treatment with T_3 lowered FT_4 and TSH in survivors but not in nonsurvivors. Nonsurvivors usually exhibited an elevated reverse T_3 level whether treated or not. T_3 treatment raised T_3 levels but did not affect mortality when compared with placebo therapy.

Burned patients were hypermetabolic despite their low thyroid hormone levels. Their metabolic rate correlated well with burn size and elevated basal levels of plasma norepinephrine and somewhat with plasma epinephrine. Severely burned patients developed a syndrome characterized by elevated sympathetic activity and metabolic rate and a depressed pituitary-thyroid axis. This response is different from the hypometabolic low T_3 syndrome of starvation but may be a frequent reaction to nonthyroidal illness.

Secondary hypothyroidism
Tertiary hypothyroidism
Septic
Terminally ill
Burn patients

**ASSESSMENT OF L-TRIIODOTHYRONINE THERAPY IN THERMALLY
INJURED PATIENTS -- THE HYPERMETABOLIC LOW
TRIIODOTHYRONINE SYNDROME IN BURNED SOLDIERS**

Many nonthyroidal illnesses (NTI), such as starvation, infection, liver disease (1, 2), kidney disease (3), malignancy (4), myocardial infarction (5), diabetes mellitus (6) and accidental burn injury (7, 8) are associated with a decrease in total and free triiodothyronine (T_3) concentration in plasma. Malnutrition (9), burn injury (7, 8), and coma due to head trauma (10) are also associated with a fall in tetraiodothyronine (T_4) level. Reduction in T_4 may signify greater severity of illness (11): patients with a low total T_4 level on admission to a medical intensive care facility had a subsequent mortality (63%), more than fourfold that of patients with

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normal T_4 . Their thyrotropin (TSH) levels were not elevated despite a reduced free T_4 index (FT_4I) and a very low total T_3 (11). It seems likely that NTI (3, 4, 10) and fasting (12) reduce thyroidal secretion in part by inhibition of TSH secretion.

Experimentally, it has been assumed that the model of fasting or starvation may be generally representative of NTI (1, 13). Underfeeding not only reduces the levels of metabolically active thyroid hormones but also diminishes noradrenergic sympathetic activity, and overfeeding produces the opposite effect on both systems (14 - 19). Thus, suppression of both the thyroid axis and the sympathetic nervous system may serve to reduce metabolic rate at a time when excess oxygen consumption and catabolic activity could be disadvantageous.

The patients in this study provide an example of a low T_3 syndrome that differs radically from the starvation model of NTI: though they had suppression of the thyroid axis, a markedly elevated basal plasma norepinephrine concentration occurred at the same time and was correlated with an elevated metabolic rate. Nonsurvivors exhibited the greatest suppression of the TSH-thyroid axis.

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PATIENTS AND METHODS

We obtained serum concentrations of thyroid hormones and TSH in 215 samples from ten surviving (SURV) patients during their recovery from thermal injury with a mean total burn size (TBS) of 49% of body surface area and in 180 samples from 20 nonsurviving (NSURV) burn patients with a mean TBS of 54% (Fig. 1). Collection of SURV samples began on postburn day (PBD) 3 and continued 1 to 3 times per week to PBD 31-83, while the patients healed. NSURV samples were obtained beginning on various PBD and 0-51 days before death. The mean of all values in each patient for a given measurement was determined. The Student t test, based on mean values for each patient was used to test for significance. Five patients from each group who could be approximately matched in SURV-NSURV pairs for TBS and serial PBD of sampling are considered separately (Table 1). In none of the studies reported in this communication did patients receive iodine or iodine-containing compounds topically or systemically.

Between the tenth and twentieth PBD, five SURV and five NSURV, in addition to five nonburned healthy controls (CONT) received a single 250 mg I.V. bolus of TSH-releasing hormone (TRH). No patients received dopamine or glucocorticoids before or during TRH stimulation. Serum samples were taken for TSH assay before and at intervals following TRH injection (Figure 1). The TSH-time curve integral (area under the curve) was computed (Table 2). Analysis of variance and the Student-Newman-Keuls test were used to compare means.

While these data were being collected, 36 men, aged 17-23 yr and burned in a single accident, were admitted. After informed consent was obtained, they were entered into a formal study of T_3 versus placebo administration on a protocol approved by the institutional committee monitoring the ethical considerations of clinical studies. Eight of these patients had small burns over 2 to 7.5% of body surface and were designated as controls (CONT), since this extent of injury is not associated with alteration in metabolic rate (20). The remaining 28 had second and third degree total burn size (TBS) of 18 to 93% of body surface area and were randomly assigned in double blind fashion to treatment with either placebo or T_3 , 200 μ g/day orally or by nasogastric tube, until their wounds were healed. This dose of T_3 was previously found necessary to replete T_3 levels in burn patients. Because four patients died while receiving either placebo (NSURV) or T_3 treatment (NSURV-TX), the data were analysed according to the five groups characterized in Table 3. Results from the placebo-treated SURV and NSURV groups were also included as part of the data of

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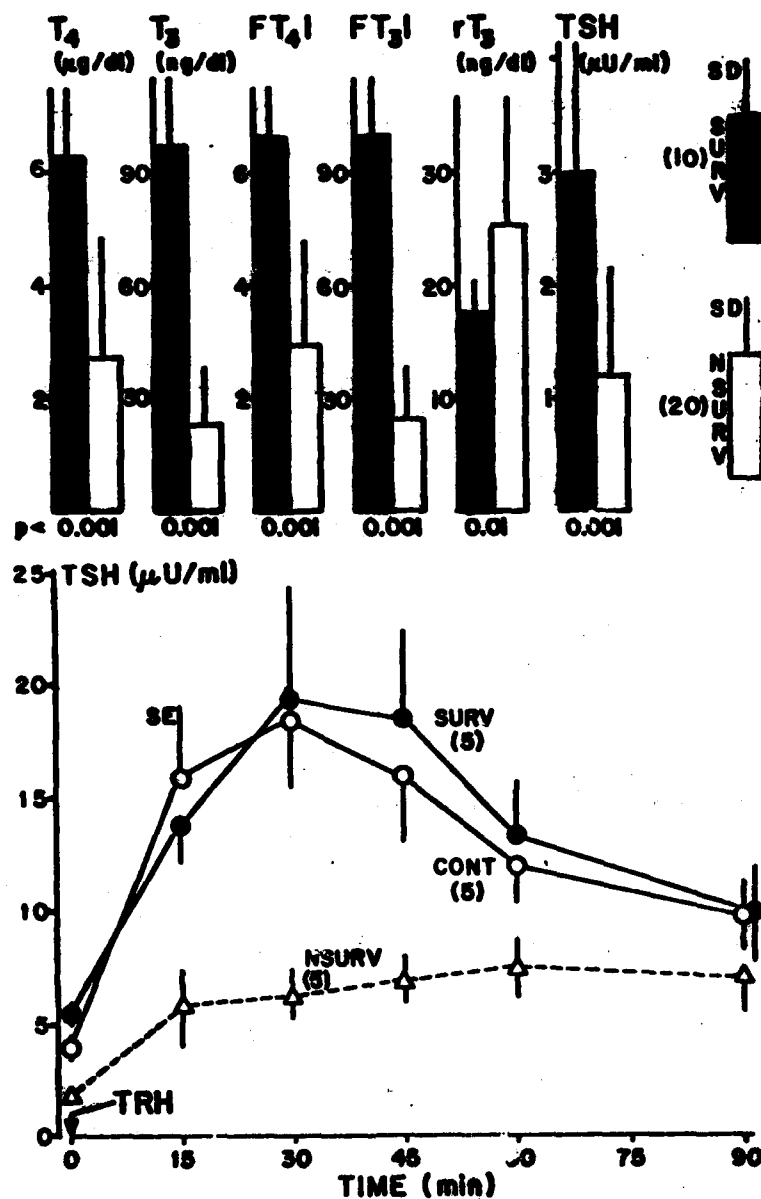


Figure 1. Serum concentrations of thyroid hormones and TSH following burn injury in survivors (SURV) and nonsurvivors (NSURV) (top panel). TSH response to TRH in representative patients and nonburned healthy controls (CONT) (bottom panel). The number of patients is indicated in parentheses in both panels.

TABLE 1. Thyroid hormones and TSH in five matched pairs of patients.

Age		Postburn Day		FT ₄ I		FT ₃ I		rT ₃		TSH	
Total Burn Size											
SURV	NSURV	SURV	NSURV	SURV	NSURV	SURV	NSURV	SURV	NSURV	SURV	NSURV
20 yrs 75%	21 yrs 70%	3	3	4.5	2.7	32	17	42	39	5.3	<0.5
		5	5*	4.0	1.9	68	29	51	28	2.0	0.8
20 yrs 80%	22 yrs 93%	3	3	3.7	3.2	41	41	40	52	2.4	1.9
		5	5	3.9	1.4	66	18	25	38	5.2	1.3
		7	7	6.3	3.5	83	22	28	43	5.3	2.6
		10	10	4.5	3.3	45	15	14	22	<0.5	<0.5
		12	12	3.8	3.9	58	<10	13	24	0.5	<0.5
		14	14*	5.8	2.6	83	27	17	11	2.6	<0.5
18 yrs 55%	22 yrs 55%	19	19	6.0	3.5	18	<10	15	35	0.7	1.0
		21	21	6.8	3.8	81	35	9	36	3.5	0.9
		24	24	6.9	1.9	74	77	10	13	3.7	0.5
		26	26†	7.9	<0.1	125	<10	9	13	4.2	1.1
		28	28†	7.1	1.5	92	<10	10	26	2.3	0.5
		31	31*†	5.9	1.2	95	27	11	46	<0.5	<0.5
20 yrs 62%	21 yrs 56%	42	45	6.8	2.5	131	51	9	27	1.3	<0.5
		45	47	6.4	3.1	151	47	13	24	1.5	<0.5
		47	49	6.5	2.4	155	45	11	26	2.1	<0.5
		49	54*	6.5	1.8	139	50	9	-	<0.5	1.7
19 yrs 53%	37 yrs 65%	19	19	8.4	2.7	75	44	19	13	2.3	0.6
		21	21	8.1	2.9	79	35	15	17	2.4	<0.5
		24	24	7.7	1.0	86	25	17	14	2.8	1.7
		26	26*†	8.1	1.3	75	41	20	23	2.5	1.9

SURV, survivors; NSURV, nonsurvivors.

*Within 24 h of death.

† Dopamine infusion.

TABLE 2. Basal FT₄I and FT₃I and TRH-stimulated TSH response in normal and burned subjects.

Group	Age (yr)	N/Sex	TBS Range (Mean)	Days Prior to Death	FT ₄ I	FT ₃ I	TSH Integral (U·min/ml)
CONT	31-40	5/M	-	-	7.9 ± 0.35	153 ± 9.5	1245 ± 208
SURV	19-54	5/M (58)	50-68	-	5.9* ± 0.7	95.0 ± 21.0	1326 ± 216
NSURV	18-63	4/M 1/F	28-68 (47)	4-7	2.9 ± 0.6	25.0† ± 6.0	579† ± 109

TBS, total burn size as % body surface; for group designations, see Figure 1.
 *p<0.05, †p<0.01; for SURV, comparison group is CONT; for NSURV, comparison group is SURV. Error terms are SEM.

TABLE 3.
Group characteristics of the T₃ treatment study.

	N	%TBS (mean)	%TBS (range)	Begin Placebo or T ₃ (PBD)	End Placebo or T ₃ (PBD)
CONT	8	4.5	2 - 7.5	-	-
SURV	10	44.3	18 - 82	3	31 - 104
NSURV	4	68.4	55 - 93	3	6 - 54
SURV-TX	10	45.3	28 - 75	3	26 - 83
NSURV-TX	4	72.9	62 - 85	3	12 - 22

TBS, total burn size as % body surface; PBD, postburn day; CONT, controls with small burns; SURV, placebo-treated survivors; NSURV, placebo-treated nonsurvivors; SURV-TX, T₃-treated survivors; NSURV-TX, T₃-treated nonsurvivors.

Figure 1 and Table 1. In this study (tables 3 and 4, figures 2 and 3), we sampled plasma for determination of thyroid hormones and catecholamines beginning on PBD 3-5, and then approximately thrice weekly, when the patients were resting in the supine position between 0500 and 0700 h. At weekly intervals, following at least an 8-h period free of caloric intake, resting metabolic rate (MR) was measured in all surviving patients. A record was kept of the total daily caloric intake and the separate intakes of carbohydrate, protein and fat.

The period of PBD 3-26 was chosen for analysis, because the major decrement in catecholamines and MR occurred by PBD 26, the control patients were available for varying periods up to this time, and all survivors received placebo or T_3 treatment during this time (Table 3). All values sampled within 24 h of dopamine or glucocorticoid administration were discarded from analysis. In one assessment of the data, the variables were considered as the mean value for each patient. But, since major changes in most variables took place over this time, the time factor was accounted in separate analyses using individual values of variables in a standard stepwise multiple linear regression program (BMPD, UCLA) performed on a PDP 1140 computer. To account for curvilinear dependent variation related to TBS and PBD, these independent variables were entered also as TBS^2 and PBD^2 into the multiple regression analysis. Other possible independent variables were added to these to determine whether they would account for variation better than the relationship to TBS or PBD. In some analyses, death or T_3 treatment were entered as independent variables.

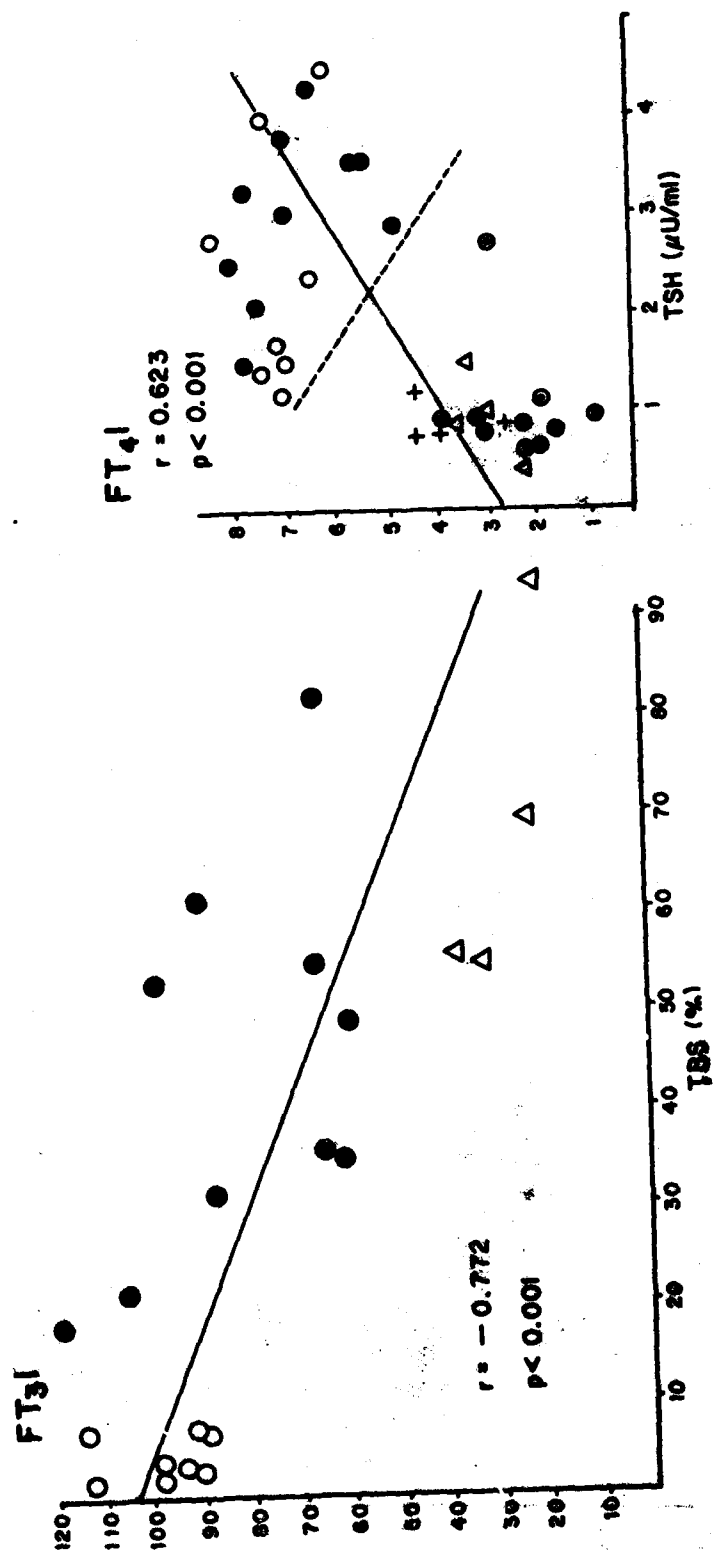
Determinations of T_4 , T_3 (Ortho), reverse T_3 (rT_3 , Serono) and TSH (Diagnostic Products) were made by radioimmunoassays with kits obtained from the manufacturers. Indices of free thyroid hormone concentration (FT_4I and FT_3I) were calculated as the product of the total T_4 ($\mu g/dl$) or T_3 (ng/dl) and the T_3 uptake (T_3U) divided by the normal calibrator T_3U provided in the kit (Ortho). The FT_4I and FT_3I were validated as indices of free hormone levels over the range observed in burn patients by determining the dialyzable fraction and the resultant free T_4 and free T_3 concentrations in 100 representative samples (Nichols Institute, San Pedro, California). Comparison of these values with respective indices yielded correlation coefficients of 0.93 (FT_4I) and 0.98 (FT_3I) (Figure 4). Plasma norepinephrine (NE), epinephrine (EPI) and dopamine (DA) were determined by radioenzymatic assay (21) as was dopamine beta-hydroxylase (22). Total plasma protein was determined according to the method of Lowry (23). Metabolic rate

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Figure 2. UPPER PANEL



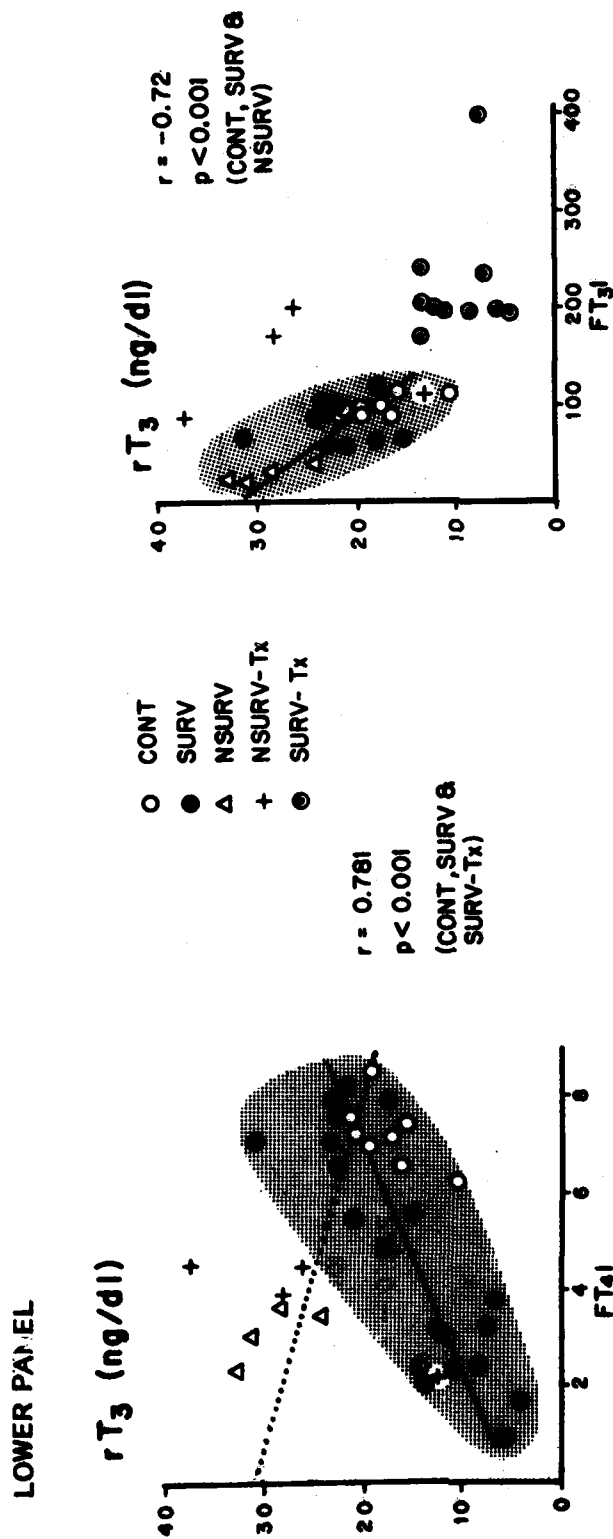


Figure 2. Relationships among thyroid hormones, total burn size (TBS) and TSH based on linear correlations of mean hormone values for each patient over postburn day 3-26. In the upper right panel, location nearer the origin indicates suppression of the pituitary-thyroid axis, and the dashed line completely separates CONT and placebo-treated SURV from the others nearer the origin. The shaded areas (lower panels) include at least all points in the regressions for groups specified in the figure. In the lower left panel, the regression depicted (solid line) is positive, because nonsurvivors are excluded. If only T_3 -treated patients are excluded, then the relationship between rT_3 and FT_4 (dotted line) is negative ($r = -0.49$, $p < 0.05$).

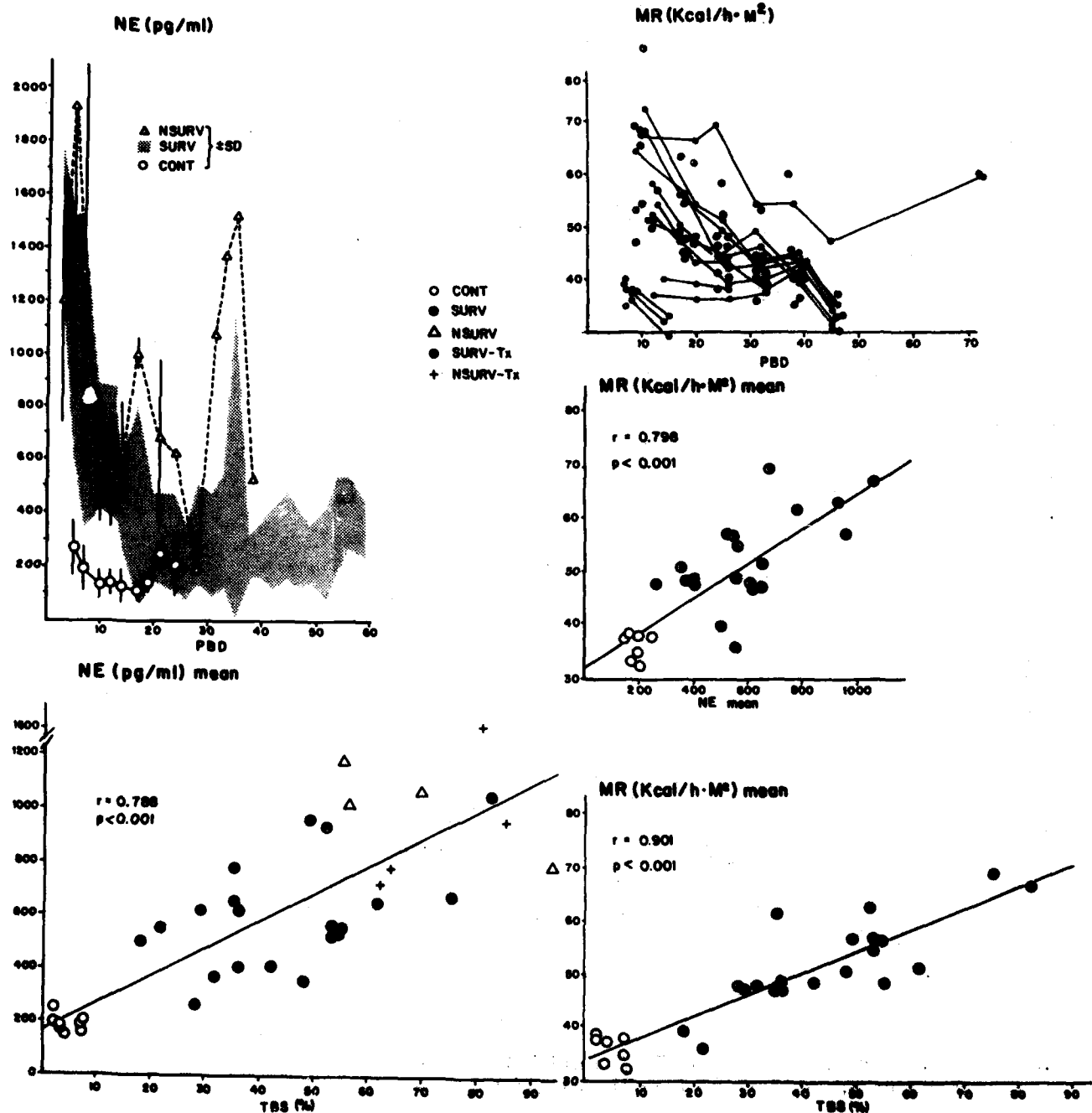


Figure 3. Elevated NE and MR in burn patients related to postburn day (PBD) and total burn size (TBS). Linear correlations are based on mean values over PBD 3-26. For group designations, see Table 3.

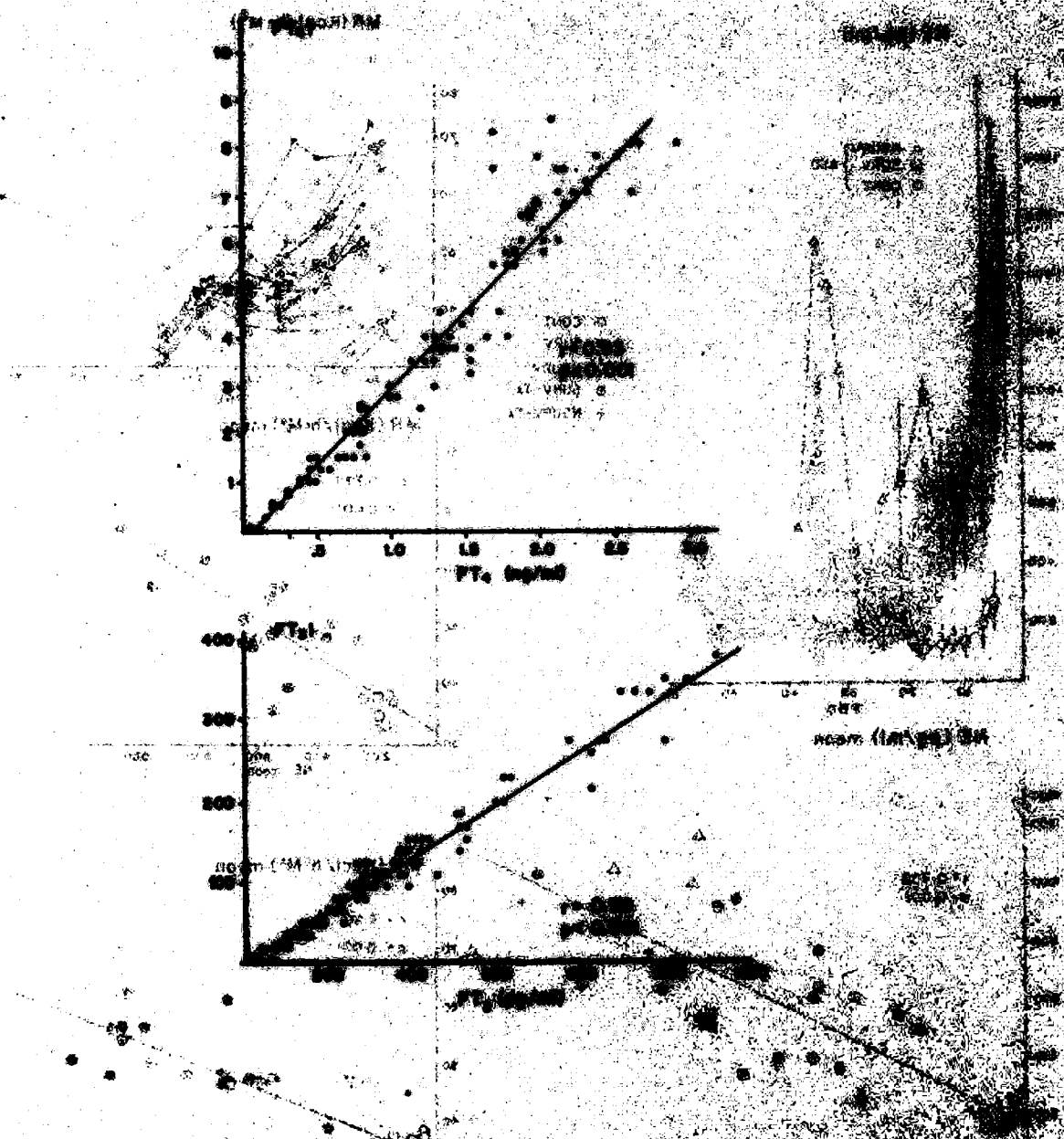


Figure 4. Correlation of FT 2 and FT 1 with concentration. The concentration data were derived from the chromatograms.

no linear correlation was observed between FT 2 and concentration. The correlation between FT 1 and concentration was also not linear. The correlation between FT 1 and FT 2 was also not linear.

(MR) was measured at ambient 31°C by indirect calorimetry based on O₂ consumption (24).

RESULTS

The pattern for total T₄ and T₃ concentration was the same as that for the respective free indices (Figure 1), and the free indices correlated closely with the free hormone concentrations in representative samples (Figure 4). Therefore, subsequent analyses were focused on the free hormone indices. In spite of reduced T₄ and T₃ concentrations, TSH was lower in NSURV than in SURV (Figure 1). This pattern in unselected patients is also seen in the set of five pairs of patients matched for burn size and times of serial hormone measurements (Table 1). TRH stimulation (Figure 1, Table 2) in SURV produced a normal TSH response, though four out of five had basal FT₃I below the lowest value for healthy controls. The response was blunted and delayed in NSURV, whose TSH concentration was higher at 60 than at 30 minutes after injection in every case. In contrast, TSH was lower at 60 than at 30 minutes after TRH injection in all CONT and SURV.

The reduction in FT₃I based on mean values for each patient was proportional to burn size in patients not treated with T₃ (Figure 2). Comparison of mean FT₄I and TSH shows that the thyroid axis was similarly suppressed in nonsurviving and T₃-treated patients (Figure 2). An inverse relationship between rT₃ and FT₄I or FT₃I can also be seen in patients not treated with T₃ (Figure 2). Multiple regression analyses showed that T₄, T₃, FT₄I and FT₃I (all p < 0.001) were inversely proportional to TBS or TBS² in placebo-treated patients. In these patients, T₄, T₃, FT₄I, FT₃I and TSH were excessively low (p < 0.01 for each) for their burn size in the nonsurvivors. T₃ treatment raised T₃ and FT₃I in survivors and nonsurvivors (all p < 0.001) and suppressed T₄, FT₄I and TSH (all p < 0.001) in survivors but not in nonsurvivors. In placebo-treated patients, higher rT₃ was associated with greater TBS (p < 0.01). T₃ treatment reduced rT₃ in surviving (p < 0.001) but not nonsurviving patients.

Patients with more extensive burns had higher NE levels and MR, particularly in the first three weeks after injury and MR is positively correlated with NE (Figure 3). NE is inversely correlated with FT₃I (p < 0.001, not shown). Multiple regression analysis showed that EPI (p < 0.001) and DA (p < 0.01) were also elevated in proportion to TBS and that nonsurvivors had elevated plasma DA (p < 0.01) but not NE or EPI concentrations out of proportion to TBS. Although T₃-treated survivors had slightly lower NE values than did untreated survivors for any given TBS and PBD (p < 0.05), NE levels were still elevated (p < 0.001) and there was no detectable effect of T₃ treatment on EPI, DA or MR.

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Interrelationships among the measured values in untreated patients were defined by considering FT_{4I} , FT_{3I} , rT_3 , TSH, a catecholamine, dopamine beta-hydroxylase corrected for total serum protein (DBH/P), or MR as the dependent variable in separate multiple regression analyses. The remaining hormones were included together with TBS, TBS^2 , PBD and PBD^2 as independent variables from which were excluded other thyroid hormones or catecholamine-related measurements if a member of the respective group was the dependent variable. TSH was considered separate from the thyroid hormone group. The program chose only those independent variables which significantly ($p < 0.05$) reduced the residual variance. The resultant equations (Table 4) indicate that while TSH and DBH/P have only weak predictive value for other variables, thyroid hormones, catecholamines, burn size and time since the burn are closely interrelated. MR was more closely related to NE than to EPI, in that the latter was not chosen as a predictor for MR. In analyses not shown, FT_{4I} , FT_{3I} , NE and MR were not correlated with total or fractionated caloric intake among SURV, indicating that differences in nutrition did not influence the metabolic parameters estimated in these patients. However, the mean total caloric intake for individual nonsurvivors was lower (NSURV, 609-1354; NSURV-TX, 537-1522 Kcal/M²/day) than for survivors (SURV, 1526-2192, SURV-TX, 1630-2256 Kcal/M²/day).

TABLE 4.

Regression coefficients of hormonal variables.

Variables, intercept, and coefficients	n	r ²
$FT_{4I} = 7.34 - 0.0003 TBS^2 - 0.002 DA + 0.001 PBD^2$	143	0.344
$FT_{3I} = 98.6 - 0.568 TBS + 0.046 PBD^2 - 0.035 DA$	143	0.417
$rT_3 = 44.2 - 3.75 PBD + 0.094 PBD^2 + 0.255 TBS$ $- 0.002 TBS^2$	143	0.540
TSH = 1.69 + 0.085 PBD	141	0.129
NE = 1425 + 22.7 TBS - 122 PBD - 0.186 TBS^2 $+ 2.92 PBD^2 - 58.2 FT_{4I}$	142	0.639
EPI = 143 + 3.0 TBS - 8.75 PBD	142	0.397
DA = 208 + 0.026 TBS^2 - 1.23 FT_{3I}	142	0.290
DBH/P = 90.1 - 0.306 FT_{3I}	141	0.037
MR = 35.1 + 0.243 TBS + 0.017 NE - 1.74 TSH $+ 0.041 DBH/P$	36	0.827

DISCUSSION

Severe burns suppress thyroid hormone levels (7, 8, 25), and this is related to extent of injury and is associated with suppression of TSH (Figures 1 and 2). Similarly, suppression of T_4 , T_3 and TSH proportional to depth of coma has been reported in patients with head trauma (10). Abnormal regulation of TSH in burn patients is also consistent with findings in fasting (12) and in other forms of NTI (3, 4). Nonsurvivors had the greatest reduction of FT_4I and FT_3I with a reduced level of TSH and also exhibited a blunted and delayed TSH response to TRH. These results are compatible with failure of brain centers controlling the thyroid axis (26), though it is conceivable that elevated DA (27) or cortisol (28, 29) might have been capable of suppressing pituitary release of TSH. In that FT_4I and FT_3I previously were found to be lower in clinically septic than in stable burn patients (7), it is possible that sepsis contributed to the excessively low thyroid axis hormone values in nonsurvivors. It is also possible that thyroid axis suppression in nonsurvivors was partly a result of a lower caloric intake. We cannot exclude the possibility that some unidentified factor also might interfere with hormone release from the thyroid, though the thyroids from our patients at autopsy have shown a thin cuboidal follicular epithelium more compatible with lack of thyrotrophic function.

Inhibited peripheral conversion of T_4 to T_3 and accumulation of the inactive rT_3 are features of other forms of NTI (1, 2, 6) and are compatible with our findings of an inverse relationship of rT_3 to FT_4I and to FT_3I in patients not treated with T_3 . Normal or high rT_3 may distinguish burn injury from the more classical forms of hypothyroidism. Factors contributing to low T_3 levels in burn patients include reduced TSH stimulation of the thyroid, probably

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diminished conversion of T_4 to T_3 , and possibly accelerated removal of T_4 and T_3 due to hypermetabolism. It has not been determined whether reduced degradation of rT_3 or elevated conversion of T_4 to rT_3 is the predominant mechanism for the elevated rT_3 levels.

Whether true hypothyroidism at the tissue level exists in the low T_3 syndrome is an unsettled issue (30, 31). T_3 treatment did not alter mortality in this study. Whether a larger experimental group might reveal some benefit of T_3 or whether there might be some benefit from T_4 therapy is not yet known. Hypothyroidism and fasting are associated with a decreased metabolic rate but burned patients are hypermetabolic. Their urinary catecholamines are elevated (20, 32-34) in proportion to metabolic rate (20, 34) as is their plasma NE (Figure 3). The hypermetabolism is blunted by propranolol (20), a beta blocker. We found MR more closely correlated with NE than EPI, suggesting a beta₁ mediation of some of the hypermetabolism. Burn patients also exhibit other signs of elevated sympathetic activity (20, 25, 34), such as elevation of heart rate, cardiac output and core temperature. The hypermetabolic low T_3 syndrome may occur in a variety of settings. Other types of trauma³ (35) and febrile illnesses (36) are associated with hypermetabolism, and febrile illnesses are associated with elevated catecholamine excretion (37) and decreased T_3 levels (38).

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33. Goodall McC: Sympatho-adrenal medullary response to thermal burn. *Ann NY Acad Sci* 150:685, 1968.

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Thus, patients with extensive burns and probably patients with other nonthyroidal illnesses develop a hypermetabolic low T_3 syndrome. Their hypermetabolism is due, at least in part, to elevated catecholamine secretion. The syndrome in burn patients would appear potentially harmful in terms of the very high levels of catecholamines or the low levels of free thyroid hormones, but attempts to alter it with T_3 administration did not greatly affect catecholamines, hypermetabolism or mortality.

PRESENTATIONS/PUBLICATIONS:

Becker RA, Vaughan GM, Seraile LG, Tucker JM, Mason AD, Jr., and Pruitt BA, Jr.: A syndrome of secondary or tertiary hypothyroidism in septic, terminally ill burn patients. Thirteenth Annual Meeting of the American Burn Association, 1981.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION#	2. DATE OF SUMMARY	REPORT CONTROLS SYMBOL DD-DRBE(AR)636	
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a. PRIMARY		61101A	3A161101A91C	00	075		
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Proceed with Security Classification Code) (U) Micromethod For Assessment of Serum Opsonic Capacity in The Burned Patient (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
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17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
Not Applicable				PRESENTING		20. FUNDS IN CURRENCY	
a. DATES/EFFECTIVE:				FISCAL YEAR		1981	
b. NUMBER:				1982		0.6	
c. TYPE:						40	
d. KIND OF AWARD:							
21. RESPONSIBLE DOD ORGANIZATION				22. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)			
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23. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME: Basil A. Pruitt, Jr., MD, COL, MC			
				POC: DA			
24. KEYWORDS (Proceed with Security Classification Code) (U) PMN leukocytes; (U) Chemiluminescence; (U) Opsonization; (U) Immunoglobulins; (U) Complement; (U) Burn Injury; (U) Human Volunteer							
25. TECHNICAL OBJECTIVE, 26. APPROACH, 27. PROGRESS (Furnish individual paragraphs identified by number. Proceeds text of each with Security Classification Code.)							
<p>23. (U) The nonspecific opsonic capacity of sera from patients following burn injury will be compared to normal control sera. Qualifications of opsonic capacity will be based upon the rate and magnitude of oxidative microbial activation as measured by amplified chemiluminescence using a set number of functional polymorphonuclear leukocytes (PMN) challenged with a set concentration of either zymosan or bacteria (<u>Staphylococcus aureus</u> or <u>Pseudomonas aeruginosa</u>). By holding zymosan and PMN leukocyte number constant, chemiluminescent activity will reflect the opsonic activity of sera. Opsonic dysfunction has been reported in severe trauma patients, and may result in increased susceptibility to infection. The present research will provide a means of monitoring immunocompetence of severely injured military patients.</p> <p>24. (U) These functional measurements will be correlated with immunologic data, such as serum complement and immunoglobulin, quantified by immunoelectrophoretic and immunodiffusion techniques.</p> <p>25. (U) 8010 - 8109. Nonspecific serum opsonic capacity has been tested in thirty-eight burn injury patients throughout their hospital-</p>							

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY 80 10 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING NA	8A. DEPTH NOTATION NL	8B. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	8. LEVEL OF COM A. WORK UNIT
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
1. PRIMARY	6T101A	3A16T101A91C	00	075			
2. CONTRIBUTING							
3. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^b (U) Micromethod For Assessment of Serum Opsonic Capacity in The Burned Patient (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA ^c 003500 Clinical Medicine							
13. START DATE 79 06		14. ESTIMATED COMPLETION DATE Cont		15. FUNDING AGENCY DA		16. PERFORMANCE METHOD C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. FUNDING (in thousands)	
A. DATES/EFFECTIVE: B. NUMBER: C. TYPE: D. KIND OF AWARD:				A. PREVIOUS FISCAL YEAR 1981 CURRENT 1982		B. FUNDING (in thousands) 0.6 0.6 20 40	
19. RESPONSIBLE DOD ORGANIZATION NAME: ^d US Army Institute of Surgical Research ADDRESS: ^e Ft Sam Houston, Texas 78234 RESPONSIBLE INDIVIDUAL NAME: Basil A. Pruitt, Jr., COL, MC TELEPHONE: 512-221-2720				20. PERFORMING ORGANIZATION NAME: ^d US Army Institute of Surgical Research ADDRESS: ^e Ft Sam Houston, Texas 78234 PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution) NAME: ^d Robert C. Allen, MAJ, MC TELEPHONE: 512-221-4311 SOCIAL SECURITY ACCOUNT NUMBER: ASSOCIATE INVESTIGATORS NAME: Basil A. Pruitt, Jr, MD, COL, MC POC: DA			
21. GENERAL USE FOREIGN INTELLIGENCE NOT CONSIDERED							
22. KEYWORDS (Precede each with Security Classification Code) ^f (U) PMN leukocytes; (U) Chemiluminescence; (U) Opsonization; (U) Immunoglobulins; (U) Complement; (U) Burn Injury; (U) Human Volunteer							
23. TECHNICAL OBJECTIVE, ^g 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) ization, and the relationship between measured opsonic capacity and clinical state has been assessed. A microtechnique for direct assessment of burn PMN leukocyte oxygenation activity using submicroliter quantities of whole blood has also been successfully developed. A portion of these results have been accepted for publication (Arch. Surg.). Currently, the techniques are undergoing modification and improvement to allow greater applicability as potential clinical laboratory techniques.							

DD FORM 1498
1 MAR 68

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

**PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT
RESEARCH**

**REPORT TITLE: MICROMETHOD FOR ASSESSMENT OF SERUM OPSONIC
CAPACITY IN THE BURNED PATIENT**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**Robert C. Allen, M.D., Ph.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MICROMETHOD FOR ASSESSMENT OF SERUM OPSONIC CAPACITY IN THE BURNED PATIENT: CHEMILUMIGENIC PROBING OF THE HUMORAL-PHAGOCYTE AXIS OF IMMUNE DEFENSE IN THE BURN INJURY PATIENT

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1980 - 30 September 1981

Investigators: Robert C. Allen, M.D., Ph.D., Major, MC
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Reports Control Symbol MEDDH-288(R1)

Primary host defense against bacterial infection is provided by an information-effector system termed the humoral-phagocyte axis of immunity. Available evidence suggests that both elements of this system for immune defense may be altered in the burn injury patient. In the present study both serum opsonic capacity and granulocyte oxygenation activity were measured in 35 burn injury patients during their course of therapy. The microbicidal action of granulocytes is effected via the metabolic generation of oxygenating agents. Introduction of chemilumigenic substrates, such as luminol or dimethyl biacridinium dinitrate, allows ultrasensitive measurement of phagocyte oxygenation activity. Serum opsonic capacity can also be assayed by measuring the rate of activation of phagocyte oxygenation activity using chemilumigenic probes. In the patients studied, alterations in granulocyte oxygenation activity were observed in individual patients in temporal association with changes in clinical condition, and sepsis was associated with a marked decrease in activity. An initial depression in opsonic capacity was noted at the time of admission of patients with major burns, > 40% total body surface. Thereafter, depression of opsonic capacity was temporally associated with sepsis in individual patients. Chemilumigenic probing provides a rapid, sensitive, and objective method for assessing the status of the humoral-phagocyte axis, and as a clinical laboratory technique, is particularly applicable for monitoring patient populations where sepsis is prevalent.

Burn Injury
Chemiluminescence
Chemilumigenic probe
Complement
Dimethyl biacridinium dinitrate

Polymorphonuclear leukocyte
Granulocyte
Luminol
Opsonin
Phagocyte

**MICROMETHOD FOR ASSESSMENT OF SERUM OPSONIC
CAPACITY IN THE BURNED PATIENT: CHEMILUMIGENIC
PROBING OF THE HUMORAL-PHAGOCYTE AXIS OF IMMUNE
DEFENSE IN THE BURN INJURY PATIENT**

Despite an expanded arsenal of antibiotics, septic complications continue to be a major contributor to morbidity and mortality in the burn patient. Such infections, commonly caused by opportunistic pathogens, imply alteration of host resistance to infection. The primary host defense against most bacterial and some fungal infections is provided by an information-effector system termed the humoral-phagocyte axis of immunity. The humoral element, composed of opsonic proteins, serves as the information mechanism which triggers a sequence of immunochemical events leading to phagocyte microbicidal activity.¹⁻³ Alterations of both elements of the humoral-phagocyte axis have been described in the burn patient.⁴⁻¹¹

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2. Allen RC. Reduced, Radical and Excited State Oxygen in Leukocyte Microbicidal activity. In: Dingle JT, Jacques PJ, Shaw IH, eds. Frontiers in Biology, Vol 48. Amsterdam, North Holland Publish Co., 1979:197-233.
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Currently available techniques for testing humoral and phagocyte function are time consuming, expensive and require a high degree of technical expertise. Chemilumigenic probes (CLP), such as luminol and dimethyl biacridinium dinitrate (DBA), have made possible the development of an ultrasensitive, rapid, inexpensive and non-destructive method for quantification of phagocyte microbicidal metabolism based on measurement of the photon emission associated with the oxygenation of these high quantum yield substrates.^{3, 12, 13} The results and interpretation of our initial research using CLP techniques for assessment of the humoral-phagocyte axis of immunity in burn patients are described in this report.

METHODS

Patient Data. The present study included eight healthy controls and thirty-five burn patients. All of the patients sustained their burn injuries in the same accident, and all were admitted to the Institute of Surgical Research on the third post-burn day (PBD). The patients were all previously healthy adult males ranging in age from 20 to 22 years. Nine patients had 2 to 18% total body surface (TBS) burns; eight patients had 21 to 36 TBS burns; ten patients had 42 to 56% TBS burns; and eight patients had 62 to 93% TBS burns. The controls included five males and three females ranging in age from 21 to 34 years. Of the 35 patients studied, 7 died. The charts of three of these non-surviving patients were reviewed, and the significant clinical changes and therapeutic interventions are presented chronologically for correlation with laboratory measurements of granulocyte oxygenation activity and serum opsonic capacity. All blood specimens were obtained between 0600 and 0700 hours. Total and differential leukocyte counts were performed on the blood specimens in order to calculate the specific granulocyte activity for each patient. Informed consent was obtained from patients and controls, and the studies were conducted in accordance with Army Medical Research and Development Command project number 3A161101A91C.

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Granulocyte Oxygenation Measurement. Whole blood obtained by venipuncture was collected in EDTA tubes for standard blood counts. A 0.1 ml aliquot was taken from the well-mixed specimen and added to a tube containing 0.9 ml of phosphate buffered saline, pH 7.2 to yield a 1 to 10 (1:10) dilution; 100 μ l of the 1:10 diluted whole blood were then added to sterile, siliconized glass counting vials (24 ml capacity) containing 1.68 ml of barbital (veronal) buffered saline with Ca^{++} , Mg^{++} , plus albumin (0.1% w/v) and glucose (0.1% w/v).¹⁴

Two chemilumigenic probes were employed. Luminol (5-amino-2, 3-dihydro-1, 4 - phthalazine-dione) was purchased from Sigma Chemical Company; a 1 mM stock solution was prepared in dimethyl sulfoxide (DMSO). Dimethyl biacridinium dinitrate (DBA; lucigenin; 10, 10'-dimethyl-9, 9'-biacridinium dinitrate) was purchased from Aldrich Chemical Company; a 1mM stock solution was prepared in H_2O . Just prior to use, the CLP stock solutions were diluted with H_2O to yield 5 μ M substocks, and 0.2 ml of either substock was added to each vial. The final CLP concentration was 0.5 μ M, and the final pH of the suspension was 7.2.

The vials were then placed in the counter, and luminescent intensity measurements were taken ever 13 min. After three measurements of prestimulation background luminescence, 20 μ l of either chemical or particulate stimulant were added per vial within 30 sec of the time zero count. The stimuli employed were both purchased from Sigma Chemical Company. A 5 mM stock solution of phorbol myristate acetate (PMA; phorbol-12-myristate-13-acetate) was prepared in DMSO. This stock was diluted with H_2O to yield a 2.5 μ M substock solution. Addition of 20 μ l of substock to the vial yielded a final concentration of 25 nM PMA. Zymosan A was suspended in normal saline (250 mg/dl), heated to 90°C for 30 min, and the cooled suspension was opsonified with an equivalent volume of guinea pig complement (250 C'H₅₀ units/ml). After 30 min incubation, the suspension was centrifuged, the serum decanted, and normal saline added to yield opsonified zymosan (Op.Zy.) 50 μ g/20 μ l.

DMSO does not influence granulocyte function at the concentration employed in these studies. Light absorption by hemoglobin presents a problem with regard to direct measurement of CL from phagocytes using whole blood specimens. In the present study this problem was sufficiently overcome by diluting the whole blood specimen 1:200 and adjusting the concentration of the CLP to provide the necessary sensitivity for oxygenation measurement.

¹⁴. Mayer MM. Complement and Complement Fixation. In: Kabat EA, Ed. Experimental Immunochimistry, Ed 2. Springfield, Charles C. Thomas, 1971: 133-162

Serum Opsonic Capacity Measurement. Blood was obtained from healthy volunteers, and the leukocyte-rich plasma isolated following heparin-dextran sedimentation. After hypotonic lysis of the remaining erythrocytes (0.2% saline for 15 sec), and two additional washes in phosphate buffered saline, total and differential counts were taken. The volume was adjusted to yield 1000 PMNL/ μ l and 25 μ l of this PMNL suspension was added to vials containing 1.75 ml of complete barbitol buffer (BBC) plus luminol as described in the previous section. The patient or control serum to be tested was diluted and a different dilution was added to each vial in order to allow titration of activity. The vials were then placed in the counter and CL intensity measurements were taken every 7 min. After three prestimulation measurements, 20 μ l (50 μ g) of zymosan suspension (unopsonified) were added per vial within 30 sec of the time zero count.

Single Photon Counting. Chemiluminescence was quantified at room temperature (22°C) using the single photon counting capacity of a Beckman LS-150 scintillation counter equipped with EMI 9829AM photomultiplier tubes, and operated in the out of coincidence mode using the tritium channel settings.¹⁵ The raw CL intensity values were converted to photons per minute by multiplying the relative counts per minute by a photon conversion factor, 14. This factor was established by calibrating the counter with a known blue photon emitter as described by Seliger.¹⁶ Values for integral CL response were calculated from the CL intensity data by trapezoidal approximation.

RESULTS

Granulocyte Oxygenation Activity. The activation of polymorphonuclear leukocyte (PMNL) metabolism associated with phagocytosis or chemical stimulation results in the generation of oxygenating agents capable of exerting microbicidal action. Introduction of bystander substrate molecules, whose oxygenation results in a high yield of electronically excited products, allows ultrasensitive measurement of phagocyte oxygenation

15. Allen RC, Stjernholm RL, Steele RH. Evidence for the Generation of an Electronic Excitation State(s) in Human Polymorphonuclear Leukocytes and Its Participation in Bactericidal Activity. *Biochem Biophys Res Commun* 1972; 47:679-684.

16. Seliger HH. Single Photon Counting and Spectroscopy of Low-Intensity Chemiluminescent Reactions. In: Peng CT, Horrocks DL, Alpen EL, eds. *Liquid Scintillation Counting, Recent Applications and Development*, Vol II, New York, Academic Press, 1980:281-319.

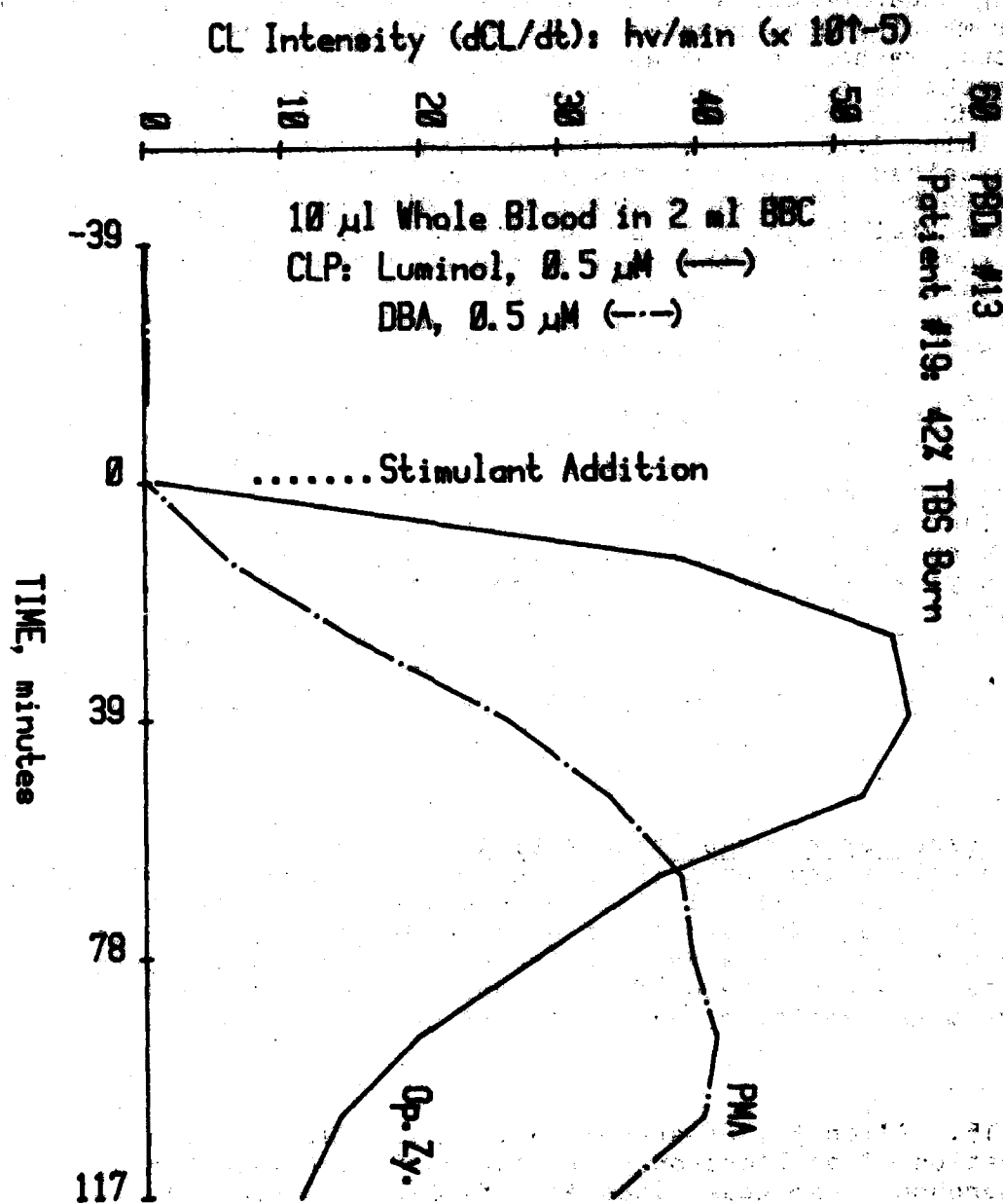


Fig 1a: Plots of intensity (part a) and integral (part b) CL data against time. The patient's whole blood specimen contained 21,800 leukocytes/ μ l with 50% segmented and 7% band neutrophils, 3% eosinophils, 4% monocytes and 36% lymphocytes. Twenty μ l of either 25 μ M phorbol myristate acetate or serum opsonified zymosan (2.5 μ g/ μ l) were added at time zero. The cumulative integral CL was calculated from the intensity data by trapezoidal approximation.

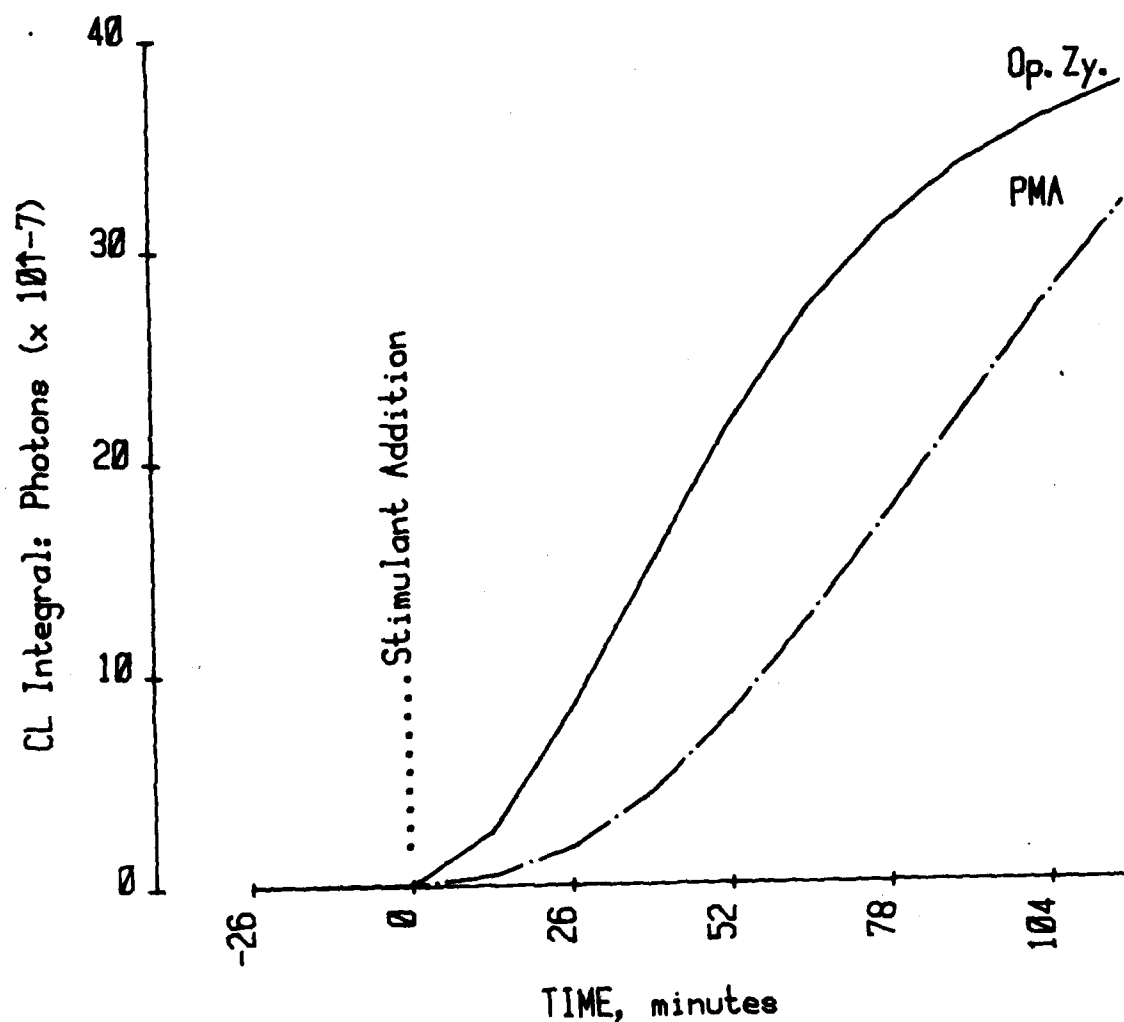


Fig 1b: Plots of intensity (part a) and integral (part b) CL data against time. The patient's whole blood specimen contained 21,800 leukocytes/ μ l with 58% segmented and 7% band neutrophils, 3% eosinophils, 4% monocytes and 28% lymphocytes. Twenty μ l of either 25 μ M phorbol myristate acetate or serum opsonified zymosan (2.5 μ g/ μ l) were added at time zero. The cumulative integral CL was calculated from the intensity data by trapezoidal approximation.

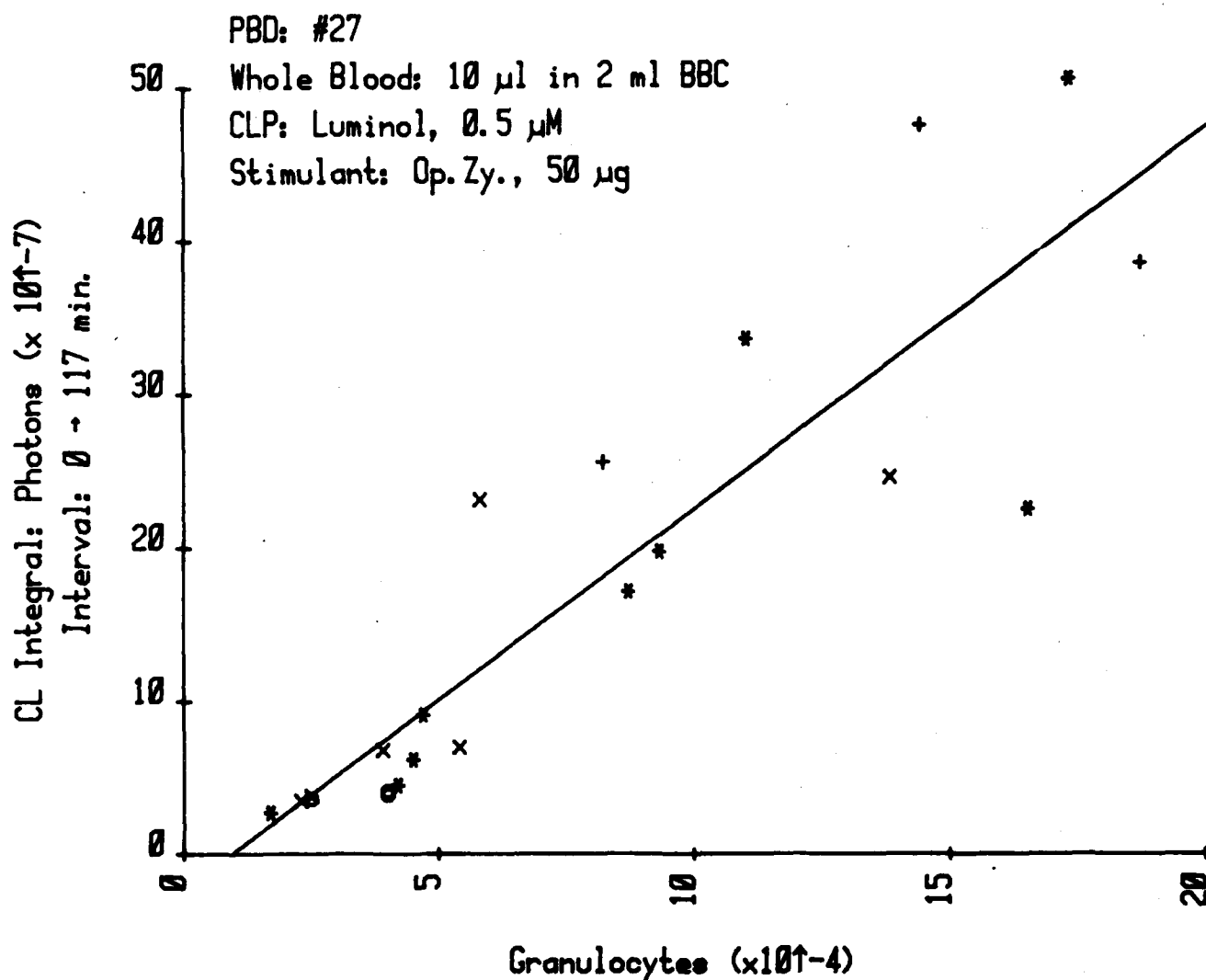


Fig 2: The integral CL response for the 2 hr interval following stimulation plotted against the total number of granulocytes in the 10 μ l specimen of whole blood tested. The granulocyte count included segmented, band, and metamyelocytic neutrophils and also eosinophils. The quantification of integral CL was as described in Fig 1. The patient specimens were divided into four groups: 1) controls plus patients with 3 to 18% TBS burns (0), 2) patients with 21 to 36% TBS burns (X), 3) patients with 42 to 56% TBS burns (*) and 4) patients with 61 to 93% TBS burns (+).

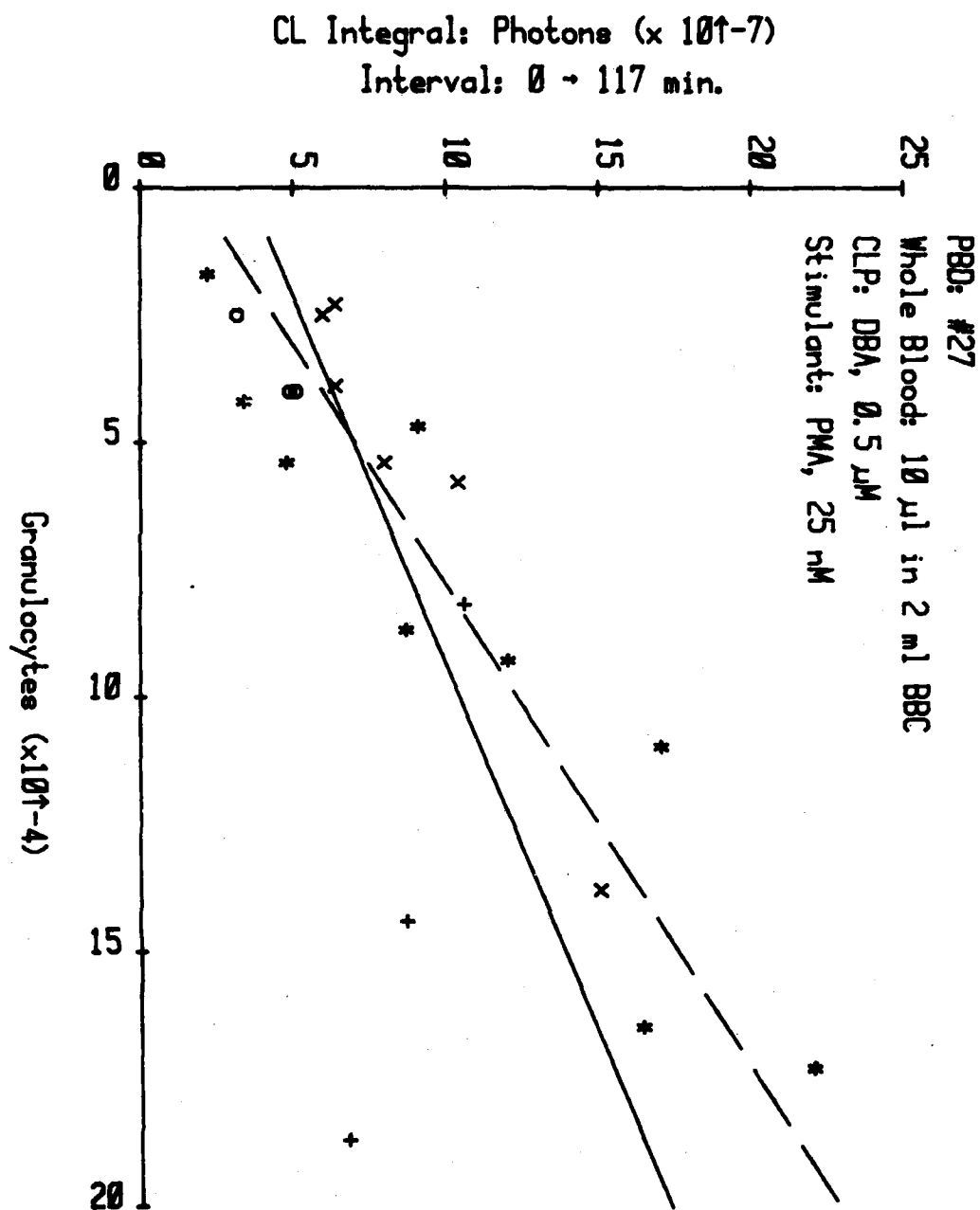


Fig 3: Plot of integral CL for the 2 hr interval following stimulation with PMA. The conditions are the same as those described for Fig 2 except that PMA was the stimulant and DBA was the chemilumigenic probe. The solid regression line is obtained when all patients are considered; the broken regression line is obtained when the 61 - 93% TBS burn patients are excluded from the calculation.

activity through quantification of the luminescence resulting from relaxation of these excited products. Appreciation of the utility of chemilumigenic probing can be gained by perusal of Fig 1a and 1b. In Fig 1a, chemiluminescence (CL) intensity (dCL/dt) is plotted against time. The portion of the curves to the left of time zero depicts prestimulation activity. The kinetics and magnitude of the CL responses from this patient's granulocytes show a pattern consistent with normal. Note that the pattern of oxygenation activity depends upon the stimulus and CLP employed. Characteristically, the granulocyte response to Op. Zy. is relatively rapid as measured by luminol oxygenation; whereas, DBA oxygenation in response to stimulation by PMA is slower but sustained. In Fig 1b the data are depicted as the integral or cumulative CL plotted against time. The integral values were calculated from the intensity data by trapezoidal approximation.

Table 1 was constructed from CL data generated by measurements as described in Fig 1. The integral CL for the two hour post-stimulation time interval was found to be a linear function of the number of granulocytes present in the whole blood specimen tested. The data of a representative experiment are depicted in Fig 2. Using Op. Zy. as the stimulant and luminol as the CLP, the total number of granulocytes in the 10 μ l blood specimen is plotted against the integral photons measured for the two-hour post-stimulation interval. As reported in table 1, for most of the experiments where Op. Zy. was the stimulus and luminol was the CLP, the coefficient of determination (r^2) was approximately 0.8, indicating a high degree of correlation. The skewing effect of a few individuals with abnormal granulocyte function was responsible for poor correlation ($r^2 < 0.8$) in some experiments. For example, on post burn day (PBD) #13, if only the surviving patients ($n=22$) are considered in the calculation, the function becomes: integral photons $\cdot 10^3/2$ hr = 2.5 (number of granulocytes) - 2.9 with an r^2 of 0.87.

Integral CL also correlated with the number of granulocytes present in the whole blood specimen when PMA was the stimulant and DBA was the CLP. However, the coefficients of determination were not as high as those obtained using Op. Zy. plus luminol. The lower r^2 values reflect the skewing effect of individual patients or groups of patients with low specific activity. This effect is graphically depicted in the plot of DBA-dependent integral CL against the number of granulocytes stimulated with PMA presented in figure 3. The r^2 for the linear regression, plotted as the unbroken line, is 0.53 when all patients and controls are considered in the calculation. However, when those patients with greater than 60% TBS burn are excluded from consideration, the equation relating CL to granulocyte count becomes: integral photons $\cdot 10^3/2$ hr = 1.0 (number of granulocytes) + 1.8 with an $n = 18$ and an r^2 of 0.87. This new plot is indicated by the broken line in figure 3.

Granulocyte oxygenation activity is reported as granulocyte oxygenation index (GOI). The GOI is calculated by dividing the actual measured granulocyte oxygenation activity of a given specimen by the calculated activity based on values obtained for controls and patients with less than 40% TBS burn. For example, patient 20 on PBD #20 had a granulocyte count of $1.3 \times 10^5/10 \mu\text{l}$ whole blood yielding 1×10^8 photons during the two hour interval following stimulation by Op. Zy. with luminol as CLP. Using the equation established for "controls plus 2-35% TBS burn" patients on PBD #20 as presented in Table 1, the granulocytes of patient 20 are calculated to yield 3×10^8 photons during this post stimulation interval. Therefore, his GOI is $1/3$ or 0.33.

Serum Opsonic Capacity. The humoral or information aspect of acute immune defense can also be tested by a modification of the CLP approach. When serum concentration is the only variable, opsonic capacity can be expressed as the rate of activation of phagocyte oxygenation activity and can be measured by chemiluminescent probing. Serum opsonification of zymosan is a measure of non-specific opsonic capacity; that is, it is considered to proceed via the alternative pathway of complement activation. The titrations of zymosan (non-specific) serum opsonic capacity for a control and a patient are depicted in Fig 4 and 5, respectively. In the figures, the integral CL responses are plotted against time. Note that the quantity and source of the serum are the only variables.

The relationship between quantity of serum and opsonic capacity is not linear, and more closely approximates the sigmoidal relationship previously established for complement hemolytic assays.¹⁴ This sigmoidal relationship is illustrated by plotting the integral CL data of Fig 4 and 5 against the quantity of serum tested as done in Fig 6. In Fig 6, the ordinate is presented as the log of the fraction: integral CL for the specimen/maximum integral CL for all specimens - integral CL for the specimen; thus, at 50% stimulation of granulocyte oxygenation activity, the ordinate value will be zero. Therefore, under the stated conditions of testing the quantity of serum required for 50% activation can be defined as a non-specific or zymosan opsonic 50 unit. For the control serum 7.8 μl were equivalent to one opsonic 50 (Op.50) unit; for the patient 22.6 μl of serum were required for one Op.50 unit. Thus the control contained 128 Op.50 units/ml serum, and the patient contained 44 Op.50 units/ml serum. The mean \pm standard deviation for seven control sera was 123 ± 19 Op.50 units/ml.

Temporal Studies of Individual Patients. The clinical changes which occurred in three non-surviving patients, as well as changes in total leukocyte and granulocyte count, and the changes across time of serum opsonic capacity and GOI, as measured by the CLP techniques described, are depicted in Fig 7, 8, and 9.

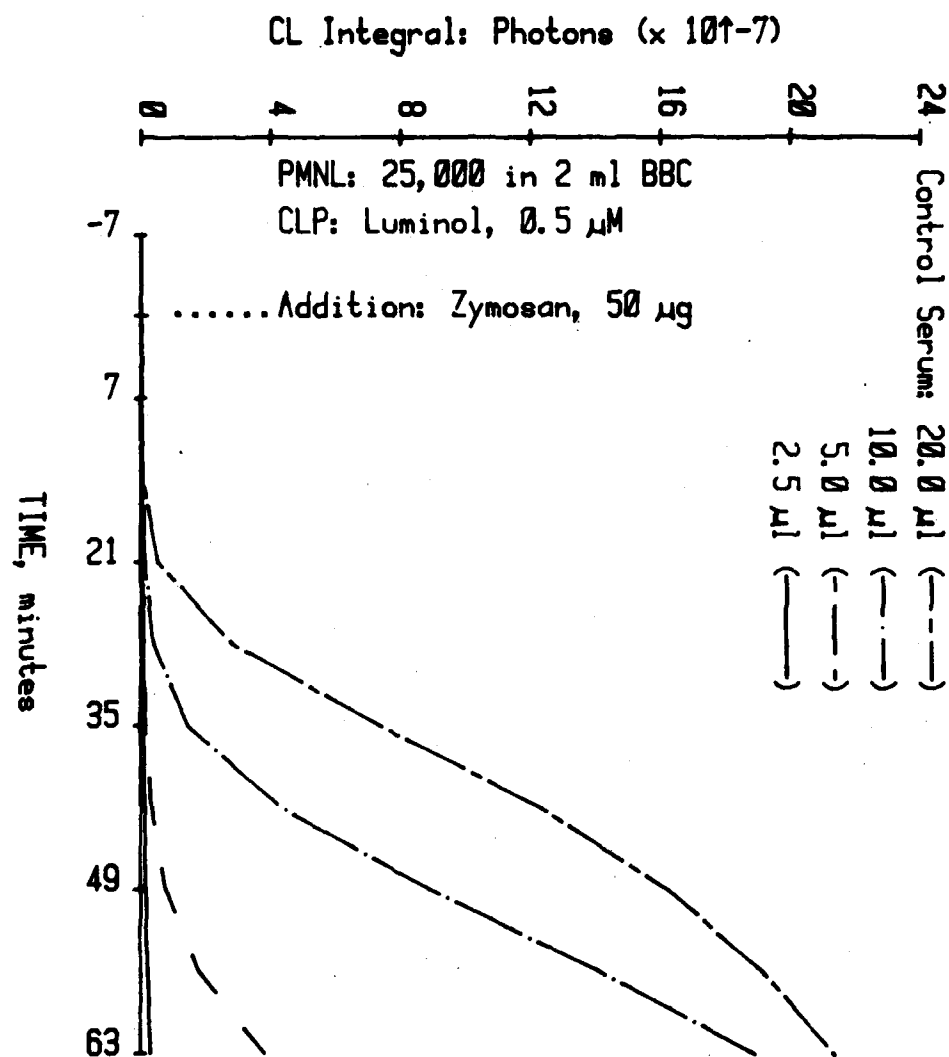


Fig 4: Plot of the cumulative integral CL against time from isolated polymorphonuclear leukocytes (PMNL) with luminol as the chemilumigenic probe. In the experiment, the only variable is the quantity or dilution of normal, control serum present per vial. Opsonification was initiated at time zero by addition of 20 μ l (50 μ g) unopsonified zymosan.

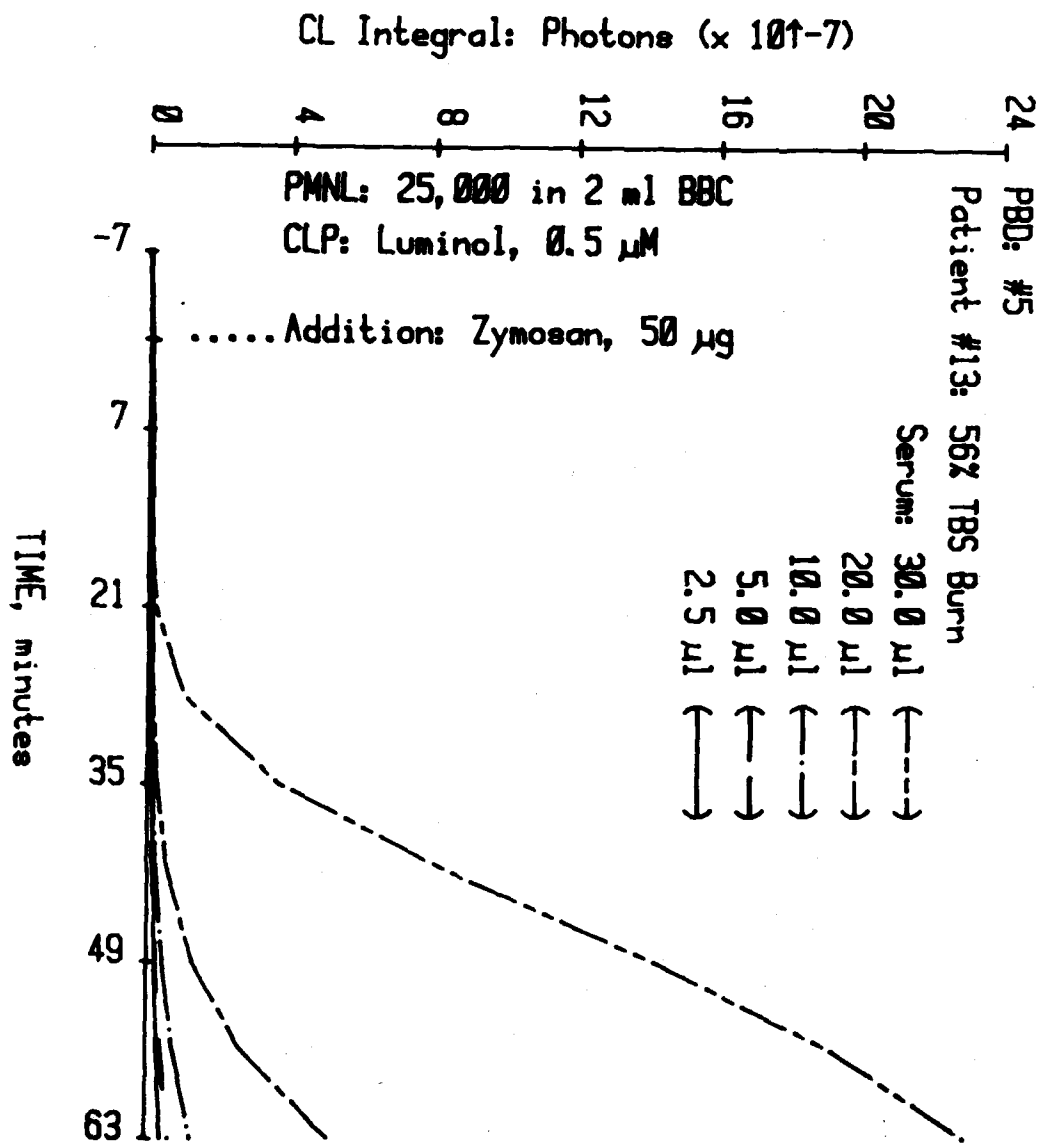


Fig 5: This plot is the same as described in Fig 4 except that the serum of patient 13 is titrated.

CL Integral: $\log [CL / (Max\ CL - CL)]$

Interval: 0 - 63 min.

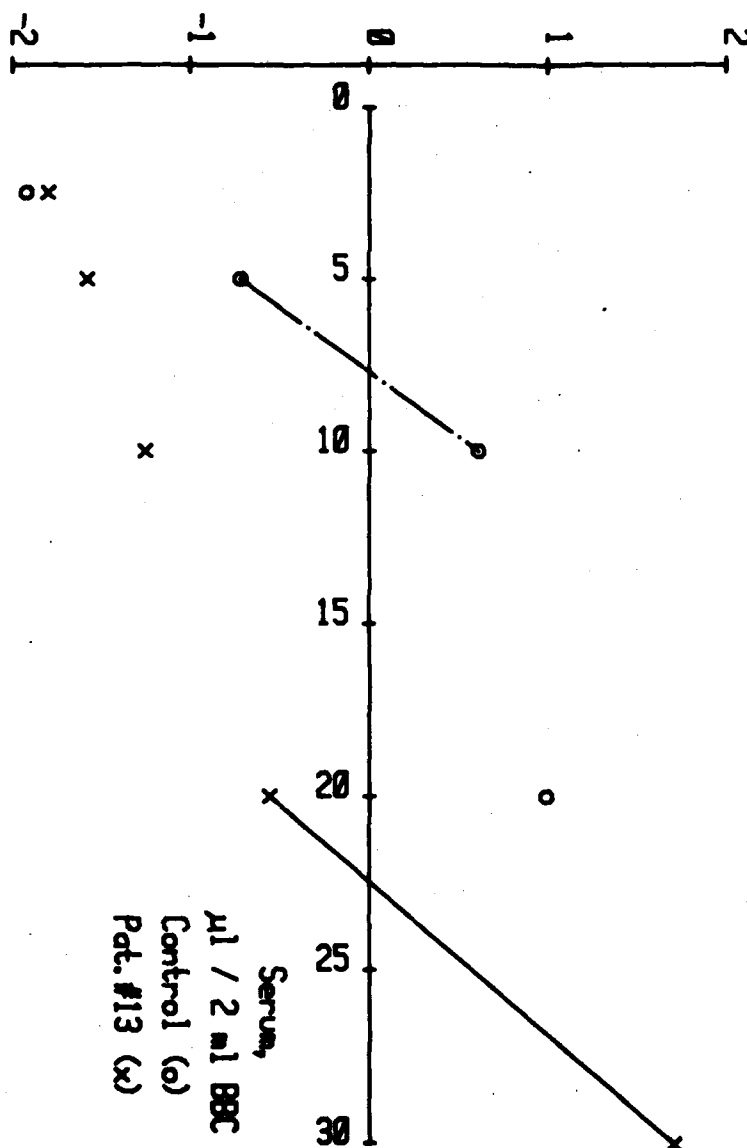


Fig 6: The integral CL response for the 63 min post stimulation interval expressed as $\log (\text{individual specimen CL} / \text{maximum CL for all specimens} - \text{individual specimen CL})$ plotted against the quantity of serum present per vial. This figure is constructed from the data presented in Fig 4 and 5. When the y-value is zero, the x-value is that quantity of serum required for 50% maximum integral CL from the granulocyte suspension.

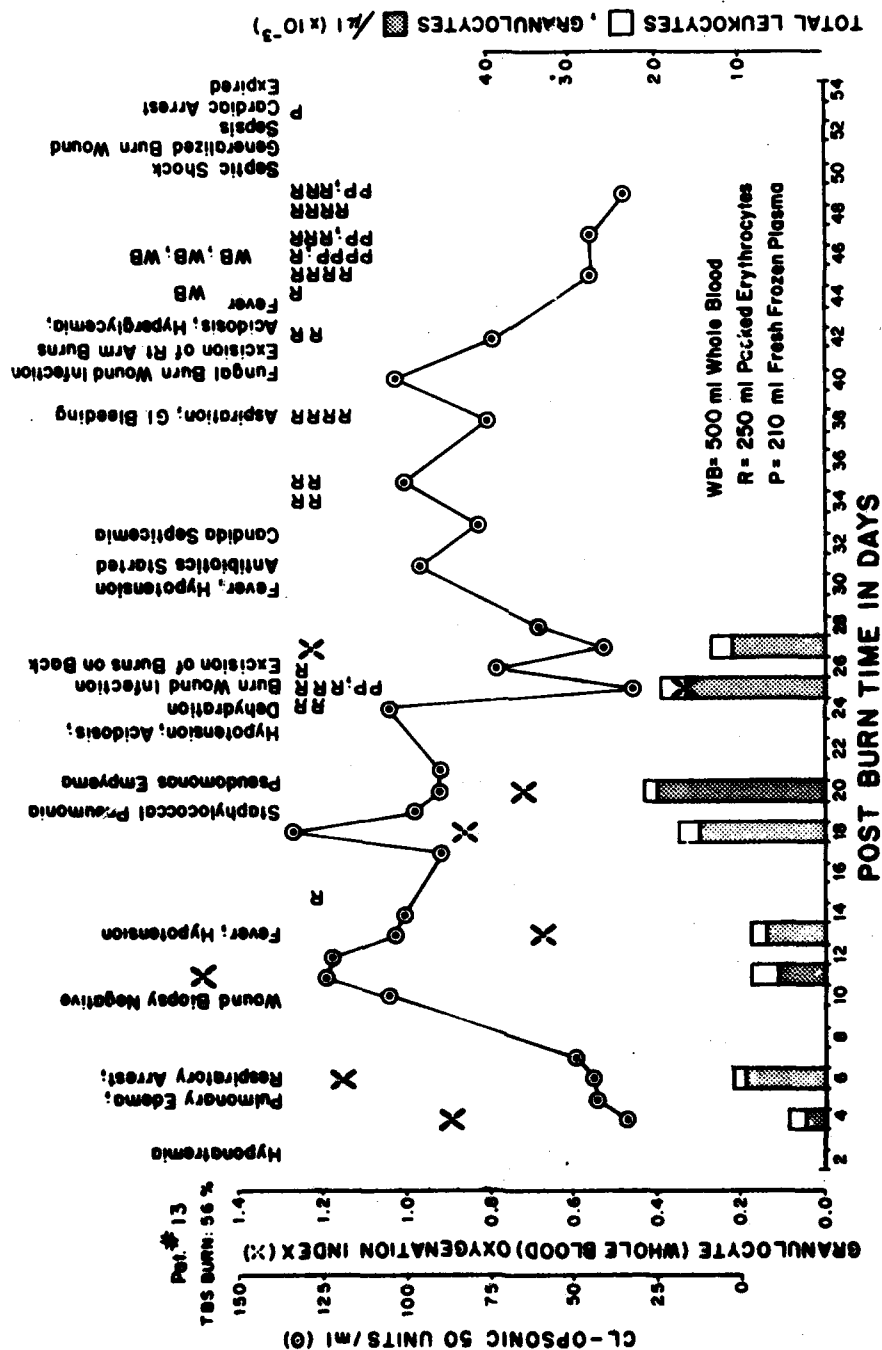


Fig 7: Temporal plot of the laboratory and clinical data for patient 13. Significant changes in clinical course are presented at the top of the figure. Transfused blood elements are represented as: WB, 500 ml whole blood; R, 250 ml packed erythrocytes, and P, 210 ml of fresh frozen plasma. The left-hand ordinate presents the value of the serum opsonic-50 capacity, and also the value of the granulocyte oxygenation index on the day tested. The right-hand ordinate refers to the bar graphic and numerically presents the leukocyte and granulocyte counts.

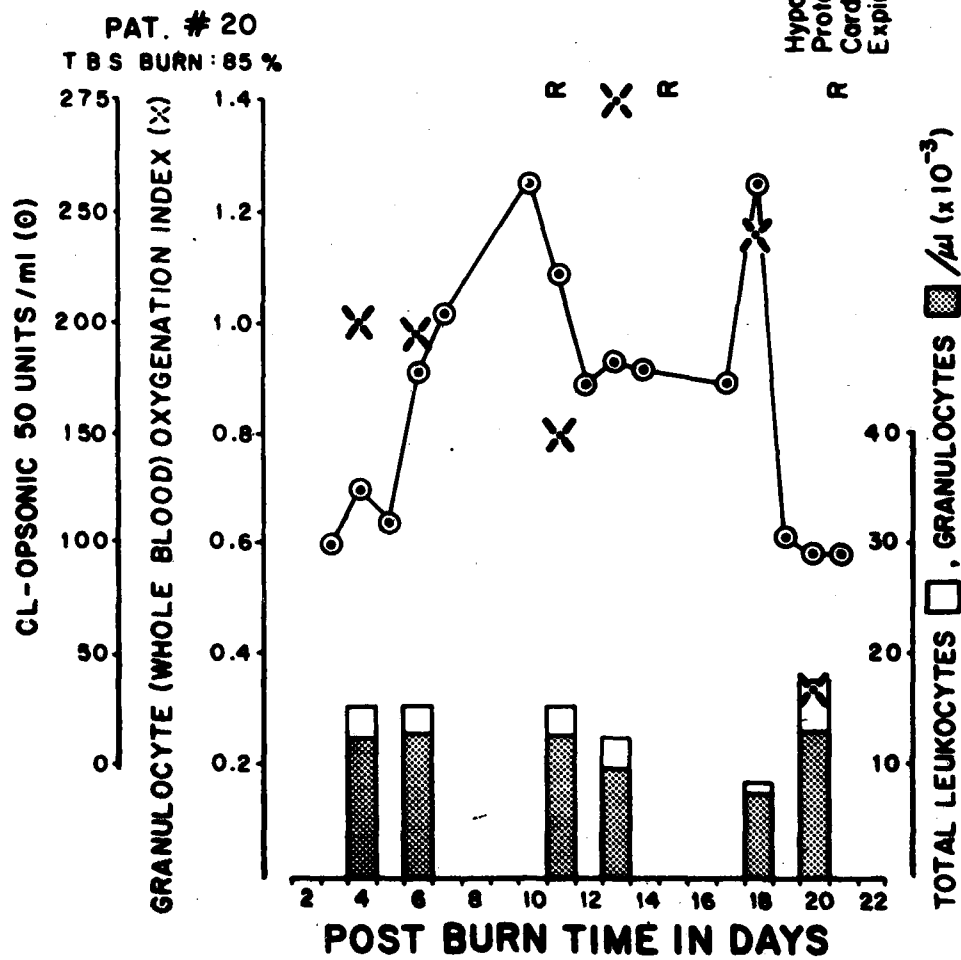


Fig 8: Temporal plot of the laboratory and clinical data for patient 20 presented as described in Figure 7.

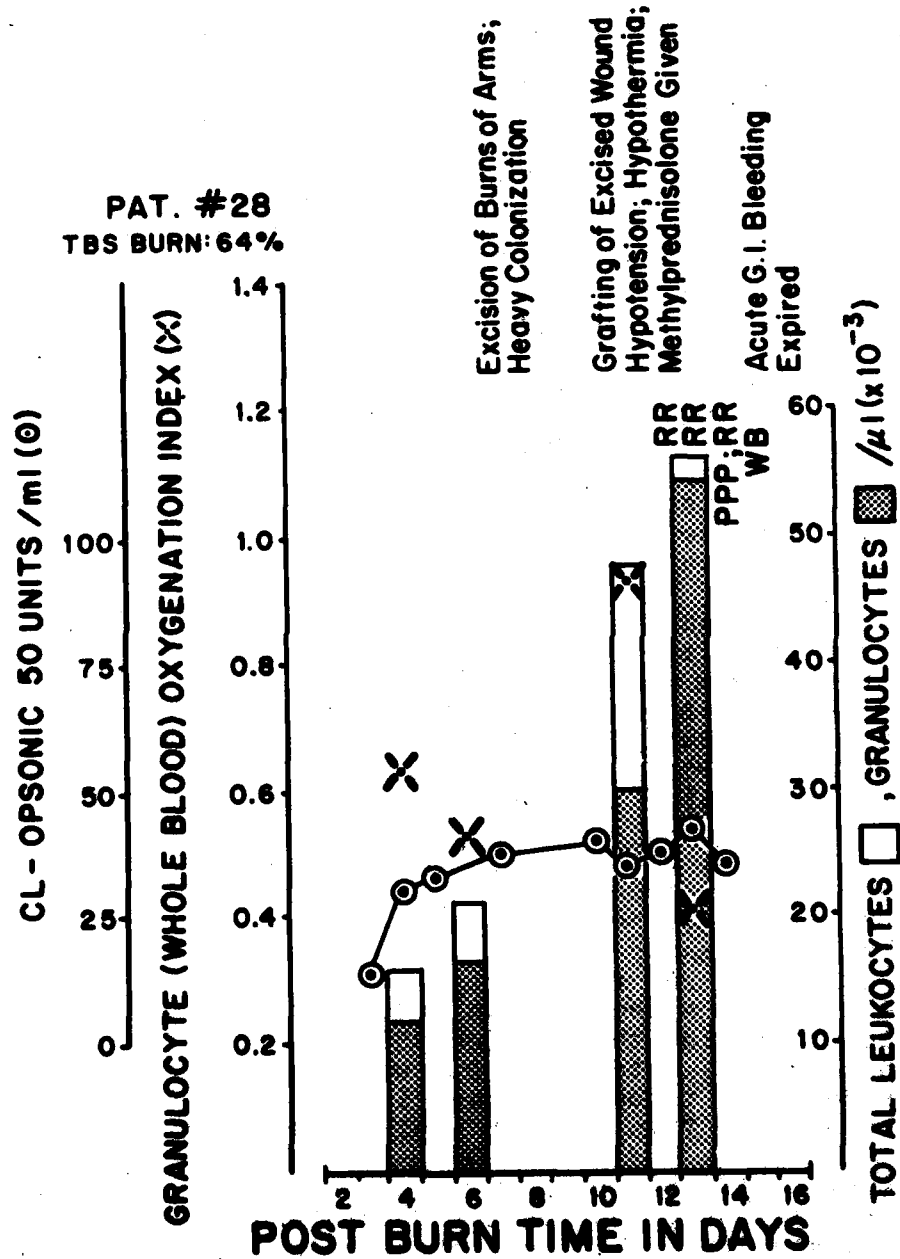


Fig 9: Temporal plot of the laboratory and clinical data for patient 28 presented as described in Figure 7.

DISCUSSION

The primary phagocyte of the humoral-phagocyte axis of acute host defense against infection is the granulocyte, and the metabolically dependent generation of oxygenating agents is essential for effective granulocyte microbicidal action.¹⁻³ The CLP techniques employed in the present study were designed to measure the oxygenation activity of stimulated phagocytes. Stimulation was effected using either serum-opsonified zymosan, a phagocytosable particulate stimulus, or PMA, a chemical stimulus. Oxygenation activity was further differentiated by use of different CLP. The luminol CL reaction is an oxidative oxygenation, and in the granulocyte, reflects myeloperoxidase-associated activity. The DBA reaction is a reductive oxygenation, and in the granulocyte can provide a measure of $\cdot O_2$ generation.¹³

Specific oxygenation activity was normal in many patients where bands and metamyelocytes accounted for greater than 70% of the total granulocyte count; therefore, segmented, band, and metamyelocytic neutrophils plus eosinophils were included in the calculation of specific oxygenation activity. Grogan has also reported that the juvenile neutrophils in burn patients have normal bactericidal capacity.¹⁰

The tabulated results of specific oxygenation measurements of granulocytes from the burn patients are presented in Table 1. Specific activity was generally lower in granulocytes from the greater than 40% TBS burn group as compared to controls plus patients with less than 40% TBS burns. However, many patients with major burn injuries had specific oxygenation activities comparable to or greater than control values. Specific activity varied with the clinical state of the patient. Figures 7, 8, and 9 depict the temporal relationships of oxygenation activity, presented as the GOI, to clinical condition in three non-surviving patients. In most instances, the GOI paralleled opsonic capacity. Measurements of GOI in patient 20 were approximately 1.0 on PBD #4 and #6, and decreased to 0.8 in temporal relation to a decrease in opsonic capacity on PBD #11. The GOI rose to greater than 1.0 on PBD #13 and #18, but on PBD #20 a profound decrease to below 0.4 was associated with a precipitous decrease in opsonic capacity and documented Proteus septicemia. This patient expired two days later.

A somewhat different pattern was observed in patient 28. GOI's were consistently low for the PBD #4 and #6 measurements in temporal association with persistently low opsonic capacity.

On PBD #13 the granulocyte count rose to 54,500/ μ l following the occurrence, and steroid treatment of, an episode of clinical sepsis as indexed by otherwise unexplained hypotension and hyperthermia. The GOI on PBD #13 was approximately 0.4, and the patient expired on PBD #16. A GOI below 0.4 was also measured in patient 13 on PBD #25 in temporal association with burn wound infection and profound depression of opsonic capacity.

These findings suggest a relationship between granulocyte oxygenation activity and state of infection. However, in the present study, frequency of sampling was limited to two specimens per week, and it is not possible to determine whether the relationship observed is one of cause or effect. Possible causes of decreased GOI are numerous and include: hormonal fluctuations, such as increased circulating levels of catecholamines and corticosteroids and decreased levels of thyroid hormone; fungal or bacterial toxemia; serum inhibitors; and circulating antigen-antibody complexes. At present there is no convincing evidence to incriminate any single mechanism.¹⁷

The serum opsonic capacities for these non-surviving patients were also measured throughout the course of hospitalization, and the results are plotted as zymosan Op.50 units/ml serum. As depicted in Fig 7, the opsonic capacity of patient 13 was markedly depressed during the first post-burn week with values ranging from 35 to 50 Op.50 units/ml serum, but by PBD #11, opsonic capacity was within the range of normal controls. After PBD #12 opsonic capacity followed a variable course in association with multiple clinical episodes of infection. A profound decrease in activity to 33 Op.50 units/ml was measured on PBD #25 in association with documented burn wound infection. During the period from PBD #28 to #40, opsonic capacity was measured intermittently with fluctuations in activity observed in association with Candida septicemia and aspiration. Following PBD #40, a progressive decrease in opsonic capacity was measured in association with fungal burn wound infection. During the following nine days, an insidious decrease in opsonic capacity paralleled preterminal clinical deterioration, and was not reversed by administration of multiple units of whole blood, packed erythrocytes, and fresh frozen plasma.

As shown in Fig 8, patient 20 had 101 Op.50 units/ml serum on PBD #4, a value only slightly below the mean \pm standard deviation (123 ± 19) of the control sera tested. By PBD #7 the

17. Ransjö U, Forsgren A, Arturson G. Neutrophil Leukocyte Function and Wound Bacteria in Burn Patients. Burns 1976; 3:171-178.

serum capacity had risen to 204, and by PBD #10, it reached a maximum of 263 Op.50 units/ml. Even though at near control levels, the serum opsonic capacity measurements on PBD #3, #4 and #5 were low relative to the patient's maximum capacity, and on PBD #19 and #20, opsonic capacity fell to a level of 97 Op.50 units/ml in association with hypotension and Proteus septicemia. Patient 28 had exceptionally low serum opsonic capacity throughout his relatively short clinical course as depicted in Fig. 9. Only 15 Op.50 units/ml serum were measured on PBD #3, and opsonic capacity remained below 43 Op.50 units/ml until he expired on PBD #16.

Relative or absolute depression of opsonic capacity was noted on admission in all three patients. Secondary depression in serum opsonic capacity was temporally associated with sepsis, and control of sepsis was associated with improvement in capacity. Initial depression may reflect the activation and consumption of complement by heat altered tissue,¹⁸ or it may reflect transeschar exudation of complement.¹⁹ Secondary depression in activity may be related to the presence of a circulating inhibitor,²⁰ or may be related to complement consumption secondary to sepsis.

The CLP techniques employed in this study are currently undergoing further developmental improvement and testing for eventual use as a routine clinical laboratory technique. Chemilumigenic probing promises to provide a rapid, sensitive, inexpensive, and objective method for assessment of both aspects of humoral-phagocyte immunity. Such information is important since timely detection of a decrease in either humoral or phagocytic function would alert the clinician to probable sepsis, and thus prompt a thorough search for the causative infection and early treatment of the septic process.

18. Heideman M, Gelin LE. Impaired Host Defense For Infections due to Complement Consumption by Tissues Changed by Heat. Burns 1979; 5:245-247.

19. Dhennin C, Pinon G, Greco JM. Alterations of Complement System Following Thermal Injury: Use in Estimation of Vital Prognosis. J Trauma 1978; 18:129-133.

20. Bjornson AB, Altemeier WA, Bjornson HS. Reduction in C3 Conversion in Patients with Severe Thermal Injury. J Trauma 1976; 16:905-911.

TABLE I

SPECIFIC OXYGENATION ACTIVITY OF GRANULOCYTES IN WHOLE BLOOD

$$\text{Integral Photons} \cdot 10^3/2 \text{ hrs} = m \cdot (\text{Number of Granulocytes}) + b$$

Post Burn		Stim: Op.Zy.; CLP: Luminol				Stim: PMA; CLP: DBA			
Day		n	m	b	χ^2	n	m	b	χ^2
Control plus 2-36% TBS Burn	4	10	3.3	-5.6	0.84	10	1.8	0.6	0.54
	11	16	3.6	-5.9	0.85	16	1.1	2.5	0.83
	13	12	3.4	-7.2	0.96	12	1.0	4.9	0.45
	18	12	2.8	-5.9	0.82	ND	ND	ND	ND
	20	13	2.6	-4.4	0.78	13	0.6	1.9	0.54
	25	13	2.3	-2.8	0.86	13	0.9	1.8	0.83
	27	9	2.0	-0.9	0.68	9	0.9	2.8	0.84
42-93% TBS Burn	4	12	2.3	-2.3	0.87	12	1.1	-0.6	0.60
	11	12	3.1	-6.2	0.78	12	1.0	-1.2	0.89
	13	16	1.1	12.2	0.65	16	0.7	-2.9	0.70
	18	13	1.8	1.0	0.65	ND	ND	ND	ND
	20	13	2.0	-2.9	0.81	13	0.5	1.3	0.48
	25	12	3.2	-8.1	0.81	12	1.5	-4.3	0.66
	27	12	2.5	-1.7	0.75	12	0.7	3.5	0.41
Control plus all Patients	4	22	2.3	-2.4	0.88	22	1.0	2.0	0.51
	11	28	3.1	-4.0	0.84	28	0.8	2.9	0.80
	13	28	1.4	6.5	0.67	28	0.7	4.9	0.59
	18	25	2.0	-0.6	0.78	ND	ND	ND	ND
	20	26	2.1	-2.2	0.82	26	0.5	2.0	0.49
	25	25	3.1	-6.8	0.83	25	1.4	-1.5	0.65
	27	21	2.5	-2.5	0.79	21	0.7	3.5	0.53

Op.Zy.: 50 $\mu\text{g}/2 \text{ ml}$; PMA: 25 nM; Luminol: 0.5 μM ; DBA: 0.5 μM ; n: number of specimens; m: slope of linear function; b: y-intercept of linear function; χ^2 : coefficient of determination; ND: not done.

These data were obtained using the conditions of testing described in Figure 1.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMRY 81 05 29	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY ^a U	6. WORK SECURITY ^a U	7. REGRADING ^a NA	8. ORGN INSTR ^a NL	9. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES: ^a		PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY		61101A	3A161101A91C	00	076		
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a (U) Mechanisms of Opportunistic Infection in Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 010100 Microbiology and 012600 Pharmacology							
13. START DATE 81 05		14. ESTIMATED COMPLETION DATE Cont		15. FUNDING AGENCY DA		16. PERFORMANCE METHOD C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE: EXPIRATION:				PREVIOUS 1981		0.2	
b. NUMBER: ^a				FISCAL YEAR		05	
c. TYPE: 4. AMOUNT:				CURRENT 1982		0.3	
d. KIND OF AWARD: f. CUM. AMT.						20	
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a US Army Institute of Surgical Research				NAME: ^a US Army Institute of Surgical Research			
ADDRESS: ^a Ft Sam Houston, Texas 78234				ADDRESS: ^a Microbiology Branch Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish DDAY if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr., MD, COL, MC				NAME: ^a Albert T. McManus, Ph.D, MAJ, MSC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-3411			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
22. REVISIONS (Precede EACH with Security Classification Code) ^a (U) Virulence Factors; (U) Plasmids; (U) Infection; (U) Toxins; (U) Antibiotics; (U) Pharmacologic Modulation; (U) Vaccines; (U) Lab Animal							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) 23. (U) Define mechanisms of microbial pathogenicity in burned soldier. Develop methods to combat specific virulence factors of opportunistic pathogens. Identify specific defects in immune defenses targeted by opportunistic pathogen. Develop methods to increase host resistance of burned soldiers to opportunistic infection. 24. (U) This project will examine both bacterial and host factors relating to opportunistic infection. A genetic approach will be used to investigate virulence mechanisms of bacteria taken from human burn infections. Isolates will be examined for the presence of extra chromosomal elements (plasmids) that might explain differences in strain virulence. Specific hypothesis about plasmids or chromosomal gene products as virulence factors will be investigated. Virulence mechanisms will be investigated in animal models. Knowledge of specific virulence mechanisms will be used to develop pharmacological, biological or physical means to disrupt microbial virulence. 25. (U) 8010 - 8109. Strains of <u>Providencia stuartii</u> isolated from wounds and blood of septic burn patients have been investigated. All isolates were multiply antibiotic resistant. Using Agarose electrophoresis techniques, two plasmids of greater than 30 million molecular weight were present. The transferability, host range and plasmid effects on virulence are being investigated.							

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PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 69 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MECHANISMS OF OPPORTUNISTIC INFECTION IN
BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1980 - 30 September 1981

Investigators:

Albert T. McManus, Ph.D., Major, MSC
Camille Denton, M.A.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH 288 (R1)

UNCLASSIFIED

ABSTRACT

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INDEPENDENT RESEARCH

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Strains of Providencia stuartii isolated from wounds
and blood of septic burn patients have been investigated. All
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Virulence Factors
Plasmids
Infection
Toxins
Antibiotics
Pharmacologic Modulation
Vaccines
Lab Animal

MECHANISMS OF OPPORTUNISTIC INFECTION IN BURNED SOLDIERS

Efforts have been initiated to establish a plasmid isolation and identification system for burn patient bacterial isolates. The purpose of this system will be to identify specific extrachromosomal genetic elements (plasmids) and their gene products that may influence patient infections and chemotherapy. Examples of such genetic elements are antibiotic resistance plasmids and their expressed specific mechanisms of antibiotic inactivation. Other possible plasmid mediated phenomena would be proposed virulence factors such as serum resistance, tissue specific attachment ligands and antigens.

Initial efforts have been directed at enteric bacteria. A genetically marked E. coli strain C-600 was kindly provided by Dr. Eric Moody from the Department of Microbiology at the University of Texas Health Science Center in San Antonio. Strain C-600 is free of the E. coli fertility factor (F⁻) and E. coli phage lambda (λ ⁻). It requires threonine, leucine and thiamine for growth (Thr, Lev, Thi), is resistant to E. coli Phage T₁ (Ton A) and is lactose fermentation negative (Lac⁻). An additional genetic marker was added at this Institute. A nalidixic acid resistance chromosomal mutant was selected by direct plating of 10^{10} E. coli C-600 onto complete growth media containing 100 μ g/ml nalidixic acid. A spontaneous mutant was isolated and this strain was designated strain C-601. Nalidixic acid resistance is relatively rare for USAISR E. coli isolates.

Broth matings of patient E. coli strains which were nalidixic acid sensitive and had antibiotic resistance patterns different than C-601 were attempted. Following mixing of a wild strain with C-601 and incubation, the cultures were diluted and plated onto selective media containing antibiotic to kill both the "donor" wild strain and all C-601 cells that had not received the selected marker. An example would be to select for carbenicillin resistance transfer from a wild strain to carbenicillin sensitive C-601 by plating mixed cultures onto growth media containing carbenicillin (25 μ g/ml) and nalidixic acid (100 μ g/ml). By design, the wild strain would be killed by the nalidixic acid, C-601 would be killed by the carbenicillin and only bacteria with resistance to both nalidixic acid and carbenicillin would survive, i.e., C-601 with R-factor with expression of carbenicillin resistance genes. The frequency of transfer can be calculated by relating the number of transconjugates to the number of donor bacteria in the original mating mixture.

Following isolation of transconjugate C-601 clones, the strains are examined for cotransferred resistance markers which were not initially selected. Successful mating data are presented in Table 1. The plasmid designation pISR has been selected to identify isolated plasmids as coming from USAISR.

**"EXAMINATION OF RECENT PROVIDENCIA STUARTII ISOLATES
FOR TRANSFERABLE ANTIBIOTIC RESISTANCE PLASMIDS"**

Reappearance of Providencia stuartii was noted during the last quarter of FY1980. This species remained endemic during this reporting period. Patient bacteriology will be reported separately in this Annual Report (Lindberg et al).

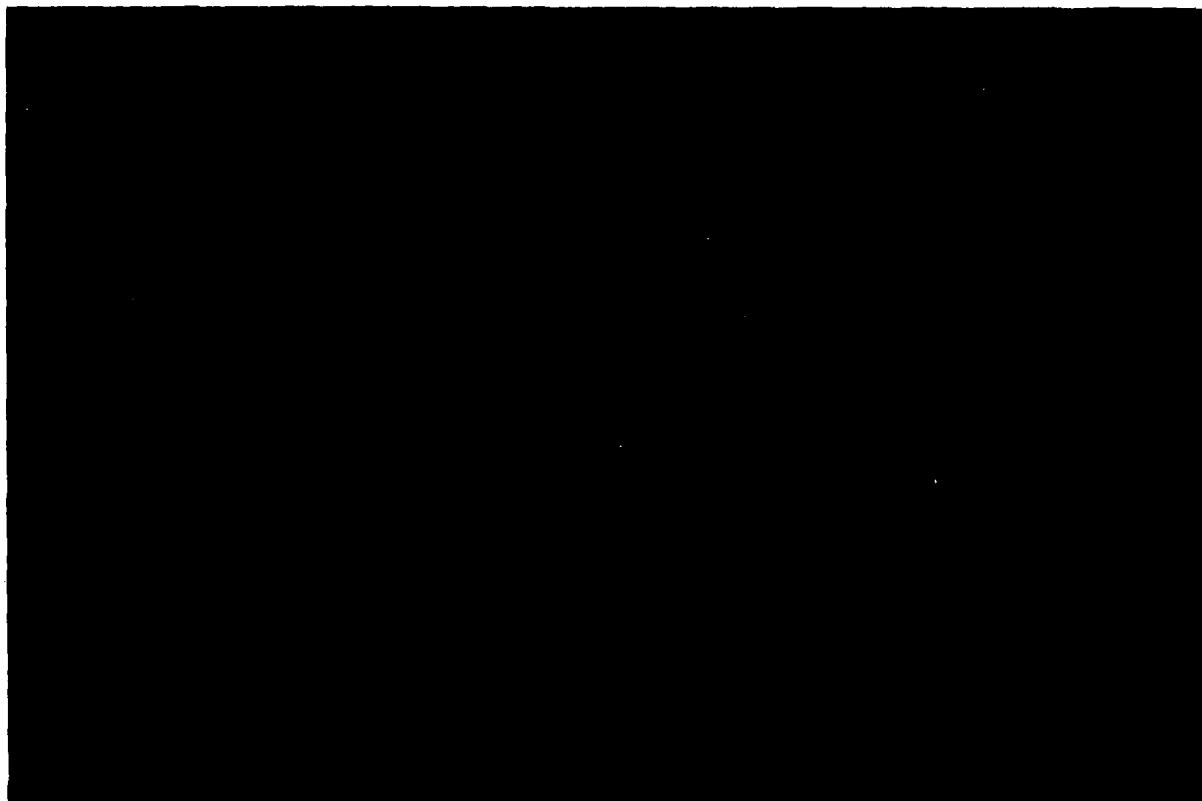
We have examined approximately 80 strains for antibiotic sensitivity to several experimental antibiotics. Antibiotic sensitivities are reported in Table 2. As can be seen, these isolates are highly resistant to aminoglycosides other than amikacin and most penicillins. The listed third generation cephalosporins were not FDA approved for human use during FY81. They are expected to be available during the next reporting period.

Transfer of antibiotic resistance from Providencia stuartii to E. coli C-601 was attempted. The endemic strain of P. stuartii is sensitive to naladixic acid. Broth cultures of P. stuartii 80111411 were mixed and collected onto a 0.45 micron millipore filter. The filter was placed on a plate of nutrient agar for 6 hours at 37°. Following incubation the filter was placed in broth and shaken vigorously to resuspend the bacteria. The broth was diluted and plated onto selective media and control antibiotics. Results are shown in Table 3. A single linkage group was found using three different selective media.

Examination of crude lysates using 0.7% agarose gel electrophoresis techniques indicated that plasmid DNA in the range of 40-50 million molecular weight was transferred from the Providencia stuartii 80111411 and E. coli C-601. (Fig. 1)

The host range and frequency of this plasmid among the bacterial ward isolates will be reported in future reports.

1 2 3 4 5 6 7 8 9 10 11 12



CR

FIGURE 1. Agarose gel electrophoresis of plasmid DNA from E. coli C601 transconjugants derived from a mating with P. stuartii 80111411. Electrophoresis was in 0.7% agarose gels at room temperature for three hours (at 100 mA) in standard borate buffer. Migration was from top (cathode) to bottom (anode). Wells 1-6 were loaded with 25 μ l sample. Wells 7-12 were loaded with 50 μ l sample. (1 & 7), E. coli C601 - parent; (2 & 8), P. stuartii 80111411 - parent; (3 & 9), progeny C-5; (4 & 10), progeny G-8; (5 & 11), progeny K-13; (6 & 12), progeny SD-17.

The parent, P. stuartii, as well as all the transconjugants each carried one large plasmid. In addition, P. stuartii as well as two of the transconjugants (C-5 & SD-17) carried a smaller plasmid.

Abbreviation: CR - Chromosomal DNA

Table 1. Antibiotic Resistance Markers Transferred from Burn Patient E. coli into E. coli strain C-601

<u>E. COLI</u> DONOR	TRANSFERRED PHENOTYPE*	DESIGNATION
046	Ap Cb	pISR1
86997	Tc	pISR2
77082914	Ap Cb Tc Sm	pISR3
77083104	Su	pISR4
77083125	Ap Cb Tc Sm	pISR5
77092624	Ap Cb Tc Su Sm Km Nm	pISR6
77081903	Ap Cb Tc Sm Km Nm	pISR7
77081903	Km Nm Sm	pISR8
77081903	Km Nm Sm Su	pISR9
77081903	Su	pISR10
77082914	Su	pISR11
77082914	Ap Cb Tc Sm	pISR12

*Resistance markers transferred with selection of a single drug marker. Nomenclature used (Novick et al. 1976 Bact. Rev. 40:168): Ap = Ampicillin, Cb = Carbenicillin, Km = Kanamycin, Nm = Neomycin, Sm = Streptomycin, Su = Sulfonamides and Tc = Tetracyclines.

Table 2. Antibiotic Sensitivity Patterns of Providencia stuartii isolated from burn patients between 1 Oct 1980 and 30 Sep 1981.

	<u>PERCENT SENSITIVE</u>	<u>STRAINS TESTED</u>
Aminoglycosides:		
Amikacin	78.5	79
Tobramycin	1.0	80
Gentamycin	0	80
Kanamycin	0	80
Neomycin	27.5	80
Streptomycin	0	80
Penicillins:		
Ampicillin	0	80
Carbenicillin	5	80
Ticarcillin	3.8	80
Piperacillin	10.	80
Mezlocillin	57.1	35
Cephalosporins:		
Moxalactam	98.8	80
Cefotaxime	95.7	47
Cefmenoxime	97.0	66
Cefsulodin	1.	80
Cefoperazone	100.	22
Other Classes:		
Polymyxin B	0	80
Colistin	0	80
Chloramphenicol	0	80
Tetracycline	0	80
Sulfonamides	0	80

Table 3. Antibiotic Resistance Markers* Transferred From Providencia stuartii to E. coli strain C-601

<u>SELECTIVE MEDIA</u>	<u>PHENOTYPE TRANSFERRED</u>	<u>TRANSFER FREQUENCY</u>
Cm(25µg/ml) + Nal (100µg/ml)	Ap Cb Cm Gm Km Hg Su Tm	4.5 x 10 ⁻⁵
Gm(25µg/ml) + Nal (100µg/ml)	Ap Cb Cm Gm Km Hg Su Tm	1.8 x 10 ⁻⁵
Km(50µg/ml) + Nal (100µg/ml)	Ap Cb Cm Gm Km Hg Su Tm	2.3 x 10 ⁻⁵
Sm(20µg/ml) + Nal (100µg/ml)	None Transferred	-----
PB(25µg/ml) + Nal (100µg/ml)	None Transferred	-----
Su(50µg/ml) + Nal (100µg/ml)	Ap Cb Cm Gm Km Hg Su Tm	9 x 10 ⁻⁵

*Antibiotic Code: Ap = Ampicillin
Cb = Carbenicillin
Cm = Chloramphenicol
Gm = Gentamycin
Km = Kanamycin
Hg = Mercury
Nal = Naladixic Acid
PB = Polymyxin B
Sm = Streptomycin
Su = Sulfadiazine = Sulfonamides
Tm = Tobramycin

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION#	2. DATE OF SUMMARY	REPORT NUMBER NUMBER	
				DA OG 6978	81 10 01	DD-DRAE(ARMED)	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. ORIGIN SYSTEM	9. SPECIFIC DATA - CONTRACTOR ACCESS	
80 10 01	K. COMP	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO A. WORK UNIT	
10. NO./CODES		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
11. PRIMARY		61101A		3A161101A91C		00	
12. CONTRIBUTING						078	
13. CONTRIBUTING							
14. TITLE (Precede with Security Classification Code) (U) Monitoring and Modification of the Metabolic and Physiologic Alterations Associated With Thermal Injury in Burned Soldiers (44)							
15. SCIENTIFIC AND TECHNOLOGICAL AREA							
003500 Clinical Medicine							
16. START DATE		17. ESTIMATED COMPLETION DATE		18. FUNDING AGENCY		19. PERFORMANCE METHOD	
78 03		81 10		DA		C. In-House	
20. CONTRACT/GRANT				21. RESOURCES ESTIMATE			
Not Applicable				22. PROFESSIONAL MAN YRS			
23. DATES/EFFECTIVE:				24. FUNDING (in thousands)			
25. NUMBER:				26. PREVIOUS			
27. TYPE:				28. FISCAL YEAR			
29. KIND OF AWARD:				29. CUM. AMT.			
30. RESPONSIBLE DOD ORGANIZATION				31. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				C, Biochemistry Branch			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Precede with U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr., COL, MC				NAME: Michael C. Powanda, Ph.D, LTC, MSC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-4106			
32. GENERAL USE				33. SOCIAL SECURITY ACCOUNT NUMBER			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
34. REVISIONS (Precede with Security Classification Code) (U) Pathogenesis; (U) Evaluation of Therapy; (U) Patient Profile; (U) Plasma; (U) Urine; (U) Enzymes; (U) Proteins; (U) Metabolites; (U) Rats							
35. TECHNICAL OBJECTIVE, 36. APPROACH, 37. PROGRESS (Precede individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To develop a profile of plasma/urine constituents which accurately reflect the severity of the thermal trauma, the presence of infection, and the healing process so as to allow objective assessment of the efficacy of therapeutic measures and to further elucidate the pathophysiology of thermal injury with the ultimate goal of lessening morbidity and mortality due to severe thermal trauma as well as hastening convalescence of soldiers who have been burned.</p> <p>24. (U) Animal and clinical studies will be run concomitantly when feasible. Animal studies, which can be rigorously controlled, will be used to test hypotheses and to expand upon the findings from patient studies. The rat burn model developed by Walker and Mason, suitably modified, will be the primary animal model employed. Initial patient studies will focus on patients who according to age and burn size, are deemed to have a 40-60% chance of survival.</p> <p>25. (U) 8010 - 8109. Of the three factors found in perchloric acid filtrates of whole blood from burned-infected rats, two (the 398 nm absorbance factor and the 355/420 nm fluorescence factor) respond predominantly to the presence of infection and are little affected by the extent of injury. These factors not only indicate the presence but also appear to reflect the severity of infection in that burned rats</p>							

DD FORM 1496

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1496, 1 NOV 66 AND 1496-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ADDRESS	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL	
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3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. RESEARCH	8. RDTM INSTR	9. SPECIFIC DATA CONTRACTOR ACCESS	10. LEVEL OF SEC
80 10 01	K. COMP	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
11. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER		WORK UNIT NUMBER		
A. PRIMARY	61101A	3A161101A91C	00		078		
B. CONTRIBUTING							
C. CONTRIBUTING							
12. TITLE (Provide with Security Classification Code) (U) Monitoring and Modification of the Metabolic and Physiologic Alterations Associated with Thermal Injury in Burned Soldiers (44)							
13. SCIENTIFIC AND TECHNOLOGICAL AREA							
003500 Clinical Medicine							
14. START DATE		15. ESTIMATED COMPLETION DATE		16. FUNDING AGENCY		17. PERFORMANCE METHOD	
78 03		81 10		DA		C. In-House	
18. CONTRACT/GRANT				19. RESOURCES ESTIMATE		20. PROFESSIONAL MAN YRS	
Not Applicable				PRESTIGE		21. FUND (in thousands)	
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C. TYPE:				1982		00	
D. KIND OF AWARD:				0.0		00	
23. RESPONSIBLE S&T ORGANIZATION				24. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				C, Biochemistry Branch			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic institution)			
NAME: Basil A. Pruitt, Jr., COL, MC				NAME: Michael C. Powanda, Ph.D, LTC, MSC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-4106			
25. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
26. KEYWORDS (Provide each with Security Classification Code) (U) Pathogenesis; (U) Evaluation of Therapy; (U) Patient Profile; (U) Plasma; (U) Urine; (U) Enzymes; (U) Proteins; (U) Metabolites; (U) Rats							
27. TECHNICAL OBJECTIVE, 28. APPROACH, 29. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with security Classification Code.)							
<p>previously inoculated with an experimental vaccine which protected 90% of the animals upon challenge with the parent strain of microorganisms had significantly lower values for these indicators than did unvaccinated burned infected rats. These two factors require the presence of both plasma and the cell fraction of blood to obtain maximal response. In contrast the 280/340 fluorescence factor responds equally to infection and injury and plasma alone is sufficient to yield a maximal response. This phase of the study is now complete.</p>							

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1498, 1 NOV 68 AND 1 NOV 71, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C- 00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

**REPORT TITLE: MONITORING AND MODIFICATION OF THE METABOLIC AND
PHYSIOLOGIC ALTERATIONS ASSOCIATED WITH THERMAL INJURY
IN BURNED SOLDIERS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**Michael C. Powanda, Ph.D., Lieutenant Colonel, MSC
John Dubois, B.S.
Ysidro Villarreal, B.S.**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE INDEPENDENT LABORATORY RESEARCH

REPORT TITLE: MONITORING AND MODIFICATIONS OF THE METABOLIC AND
PHYSIOLOGIC ALTERATIONS ASSOCIATED WITH THERMAL INJURY
IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 October 1980 - 30 September 1981

Investigators: Michael C. Powanda, Ph.D., Lieutenant Colonel, MSC
John Dubois, B.S.
Ysidro Villarreal, B.S.

Reports Control Symbol MEDDH-288(R1)

Three factors can be detected in perchloric acid filtrates of whole blood taken from burned-infected rats. One of these factors has a maximal absorbance at 398 nm; the other two factors fluoresce, one at 340 nm (excitation 280 nm), the second at 420 nm (excitation 355 nm). The 398 nm and the 355/420 factors respond primarily to the presence of infection and are only affected by the extent of injury during the first 48 hours postinjury. These factors not only indicate the presence but also appear to reflect the severity of infection. Burned-Pseudomonas-infected rats which had previously been inoculated with an experimental, ribosomal, strain-specific vaccine which protects > 90% of such animals against death had significantly lower values for both of these indicators than did unvaccinated burned-infected rats, all of which would die within 7-8 days. In contrast the values of these indicators in burned-infected rats which were treated with immune serum which increases survival time but does not lessen mortality were not different from those of untreated-infected animals. The 398 nm and 355/420 factors require the presence of plasma and cells for their generation. It appears that complement is not involved in the generation of the biochemical indicators and that the cells required are the erythrocytes. The plasma must come from burned-infected animals, the cells may come from unburned, uninfected animals, thus allowing for the possibility of retrospectively analyzing stored plasma samples for the presence of these indicators. The 280/340 factor responds equally well to infection and injury and plasma alone is sufficient to yield maximal response. This factor appears to be a good indicator of the presence of inflammation, irrespective of etiology. Preliminary studies suggest that all these factors are proteinaceous and have molecular weights in the 25,000-75,000 dalton range. A clinical evaluation of the applicability of these indicators to burn patient care is under way.

Thermal injury
Infection
Rats

Indices of infection
Plasma
Cells

MONITORING AND MODIFICATIONS OF THE METABOLIC AND PHYSIOLOGIC ALTERATIONS ASSOCIATED WITH THERMAL INJURY IN BURNED SOLDIERS

INTRODUCTION

The treatment of severe thermal trauma is very often complicated by infection which occurs readily in such patients. The loss of the skin barrier and the extensive metabolic and physiologic alterations in burn patients renders detection of infection more difficult and may allow colonization to be mistaken for infection. Three factors have been found in perchloric acid filtrates of whole blood taken from burned-infected rats, two of which appear to be useful indicators of the presence of infection and the third a measure of the presence of inflammation, irrespective of etiology (1,2). The following presents the data that the 398 nm absorbance factor and the λ : 355, λ em 420 fluorescence factor not only denote the presence of infection, but also reflect the severity of infection. Included is the evidence that these two factors require both plasma and cells for their generation as well as the attempts to ascertain which cell population is involved.

METHODS AND MATERIALS

Rats used in these studies were obtained either from Holtzman or from Timco. The standard burn model of Walker and Mason (3) was used, and burn size varied as needed. Pseudomonas aeruginosa strain 12-4-4 was used to infect the burned rats. Partial correlation and multivariate regression was accomplished using the BMDP biomedical computer programs.

RESULTS AND DISCUSSION

In order to assess the value of the newly discovered biochemical indicators of infection as compared to other chemical indices of infection (4,5), the following study was carried out using fed and fasted rats. Twenty-four and 48 percent total body surface burns were produced, and one-half of each group of burned rats were inoculated with Pseudomonas.

1. Powanda MC, Dubois J, Villarreal Y, Walker HL, Pruitt BA Jr: Detection of potential biochemical indicators of infection in the burned rat. J Lab Clin Med 97:672-679, 1981.
2. Powanda MC, Dubois J, Villarreal Y, Walker HL, Pruitt BA Jr: Indices of infection and/or inflammation in the burned and burned-infected rat (Abstract). Fed Proc 40:919, 1981.
3. Walker HL, Mason AD Jr: A standard animal burn. J Trauma 8: 1049-1051, 1968.
4. Powanda MC, Cockerell GL, Moe JB, Abeles FB, Pekarek ES, Canonico PG: Induced metabolic sequelae of tularemia in the rat: Correlation with tissue damage. Am J Physiol 229:479-483, 1975.
5. Berendt RF, Long GC, Abeles FB, Canonico PG, Elwell MR, Powanda MC: Pathogenesis of respiratory Klebsiella pneumoniae infection in the rat. Bacteriologic and histologic findings and metabolic alterations. Infect Immun 15:586-593, 1977.

At 2 and 4 days, rats were killed and the blood analyzed for the biochemical indicators and the plasma for selected proteins. Figure 1 depicts the results of these analyses. On day 2, injury caused an increase in all indicators, save for albumin which decreased, while injury plus infection produced even greater changes. By day 4, the only significant increase in OD 398, λ ex 355/ λ em 420 and α_2 -macroglobulin were the result of infection; there was little effect of injury. Seromucoid concentration as well as λ ex 280/ λ em 340 increased while albumin decreased in response to injury alone, with somewhat greater changes due to injury plus infection. Fasting did not appreciably alter the extent of change in any of these indicators of infection and/or inflammation.

Table 1 summarizes the correlations between alterations in the indicators and burn size or the presence of infection. It is apparent that injury is a major factor in changes in concentration of these indicators on day 2, but on day 4, it is evident that the increases in α_2 -macroglobulin, OD 398 and fluorescence 355/420 are primarily in response to the presence of infection. This point is made clearer by Table 2, which shows the significance levels for the regression coefficients in Table 1.

Table 1. Correlation of Dependent Variables with Burn Size or Presence of Infection

	Day 2		Day 4	
	Burn Size	Infection	Burn Size	Infection
Albumin	-.830	-.586	-.807	-.540
Seromucoid	.849	.655	.588	.844
α_2 -macroglobulin	.655	.592	.391	.723
OD 398	.621	.648	.304	.805
280/340	.793	.681	.493	.793
355/420	.689	.665	.334	.867

Table 2. Significance Levels for Regression Coefficients

	Albumin	Seromucoid	α_2 -MFP	OD 398	280/340	355/420
Day 2: Burn size	.001	.001	.001	.001	.001	.001
Infection	.001	.001	.001	.001	.001	.001
Day 4: Burn size	.001	.001	.011	.070	.001	.014
Infection	.001	.001	.001	.001	.001	.001

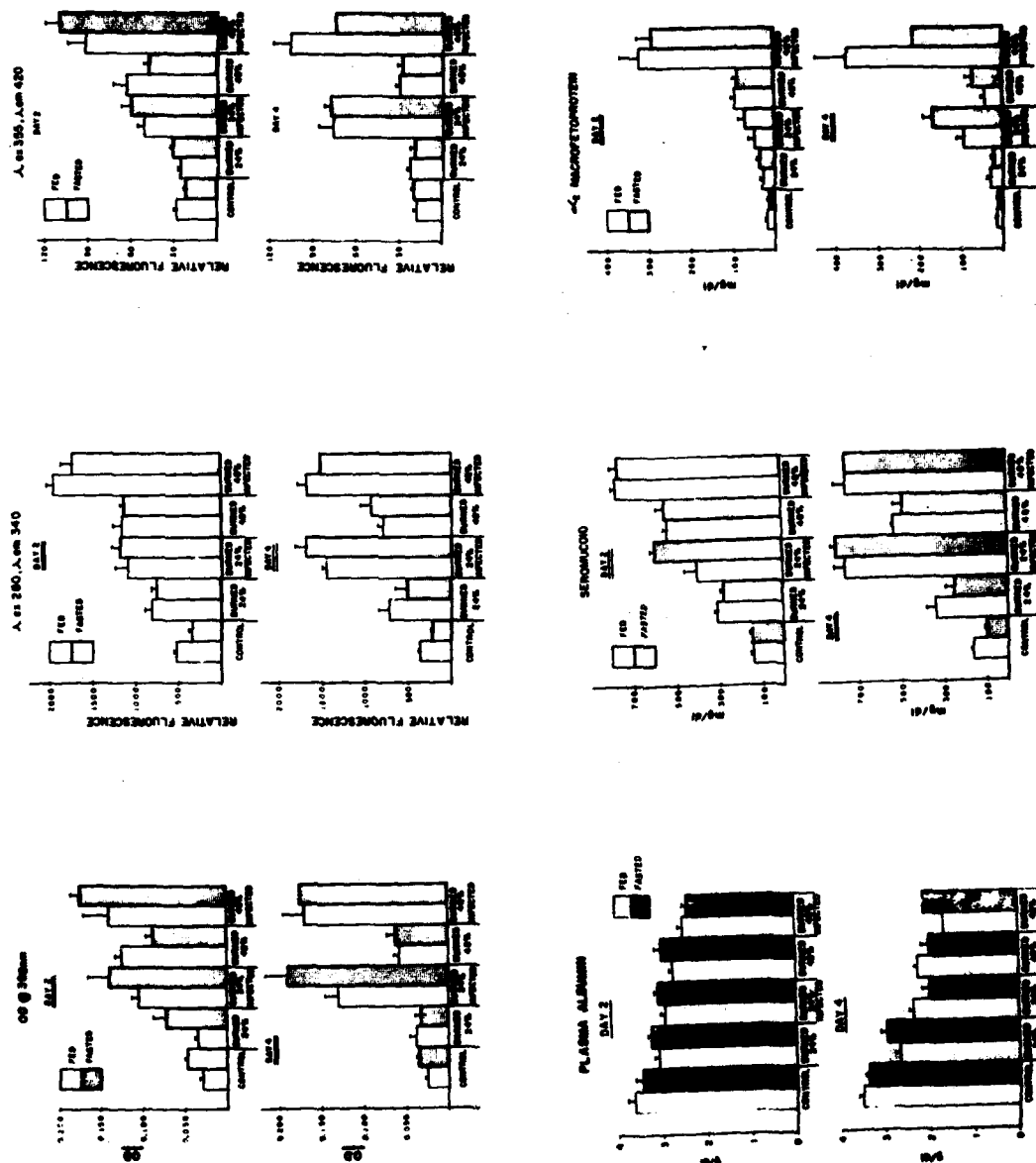


Figure 1. Effect of thermal injury, infection and fasting on selected indicators of infection and/or inflammation.

In order to assess whether the biochemical indicators of infection reflected the severity as well as the presence of infection, groups of rats were vaccinated or inoculated with immune serum and then challenged with strain 12-4-4 *Pseudomonas*. Table 3 shows the effect of vaccination with a strain-specific ribosomal vaccine on the generation of biochemical indicators. The vaccine protects > 90% of the burned-infected rats against death, while nonvaccinated burned-infected rats die between 7 and 10 days. Vaccination reduces the response of the 398 and 355/420 factors on day 5 and entirely abolishes the 398 response by day 7, while reducing the 355/420 response to 50% of the nonvaccinated rats. The 280/340 is unaffected by vaccination and seromucoid concentration only mildly suppressed. In contrast, treatment with immune serum which only lengthens the time to death but does not prevent it does not significantly inhibit the production of the 398 and 355/420 factors (Table 4).

Table 3. Effect of Vaccination with a Strain-Specific Ribosomal Vaccine on Generation of Biochemical Indicators

	Day 5				Day 7			
	OD 398	Fluorescence 280/ 340	Sero- 355/ 420	mucoid mg/dl	OD 398	Fluorescence 280/ 340	Sero- 355/ 420	mucoid mg/dl
Control	.147 ±.006	602 ± 20	40 ± 2	518 ± 25	.166 ±.015	482 ± 31	53 ± 5	498 ± 24
Burned-infected	.616 ±.050	1367 ± 51	229 ±19	1227 ± 96	.493 ±.068	1358 ±106	298 ±30	1102 ±102
Burned-infected once vaccinated day -7	.499 ±.109	1325 ±105	195 ±41	916 ± 81	.191 ±.010	1433 ±147	146 ± 1	902 ± 46
Burned-infected twice vaccinated days -7, -2	.334 ±.031	1350 ± 76	135 ±11	867 ± 56	.164 ±.030	1408 ± 94	147 ±24	946 ± 78

n = 6; mean ± SEM

Difficulties in stabilizing the perchloric acid filtrates of whole blood led us to further investigate the localization of the biochemical indicators. Table 5 shows that the OD 398 factor exists neither in the plasma nor the cell fractions but only when plasma and cells are mixed together. This is essentially true also for the 355/420 fluorescent factor. In contrast, the 280/340 fluorescent factor is found in plasma but not in cells, and in fact the addition of cells appears to interfere with its determination. Figure 2 displays the results of varying the concentration of plasma

in the presence or absence of a constant amount of cells. The appearance of the OD 398 factor and the 355/420 fluorescent factor varies as a function of the amount of plasma present as long as cells are included. There is little 398 nm material detectable in the absence of cells, but there appears to be some 355/420 fluorescence even in the absence of cells. This may be due to factors released from the *Pseudomonas* in the infected animals. The 280/340 factor also varies as a function of plasma concentration, but the presence of cells serves to interfere with its detection.

Table 4. Effect of Treatment of Animals with Immune Serum on the Generation of Biochemical Indicators of Infection

	Day 4			Day 7		
	OD 398	Fluorescence 280/340	355/420	OD 398	Fluorescence 280/340	355/420
Control	.110 ±.008	617 ± 17	38 ± 2	.121 ±.013	317 ± 60	28 ± 1
Burned-infected	.539 ±.013	1667 ± 76	169 ±12	.564 ±.063	1367 ±117	177 ±10
Burned-infected pre-immune serum	.561 ±.054	1558 ± 84	198 ± 5	.503 ±.045	1617 ±106	162 ±11
Burned-infected immune serum	.407 ±.043	1375 ± 76	133 ±14	.453 ±.072	1633 ±136	150 ±12

n = 6; mean ± SEM; sera given i.p. on day 0

Table 6 reaffirms the finding that plasma plus cells are required for the generation of the 398 and 355/420 factors and indicates that heating the plasma at 56° C for 30 minutes, which should inactivate complement, does not prevent the formation of these two factors nor does it eliminate the 280/340 factor. Furthermore, adding perchloric acid to the plasma and cells separately and then mixing the two together still allows for some production of the 398 and 355/420 indicators.

Table 7 clearly demonstrates that it is the source of plasma, not of cells, which is critical in the generation of the biochemical indicators. Plasma from control or burned rats when mixed with cells from burned-infected rats does not promote generation of indicators. However, plasma from burned-infected rats when mixed with cells even from control rats produces the 398 and 355/420 factors. The possible advantage of this finding is that retrospective analyses of stored plasma samples from animals (and, one hopes, patients) suspected of having been infected can be carried out using normal cells, perhaps even from a different species.

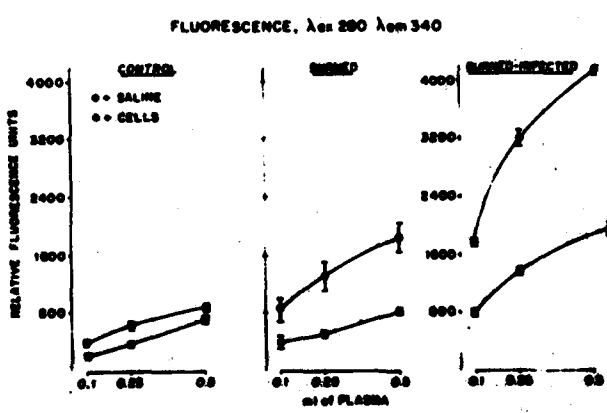
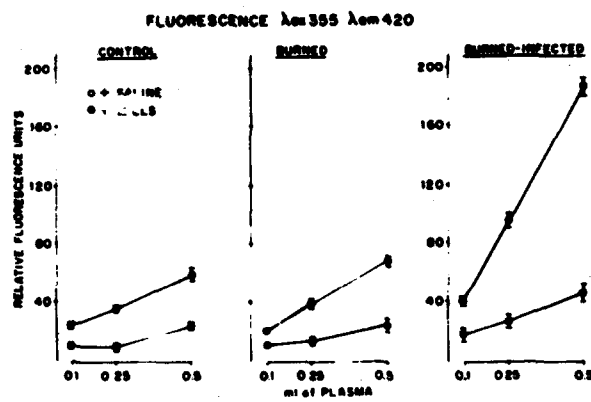
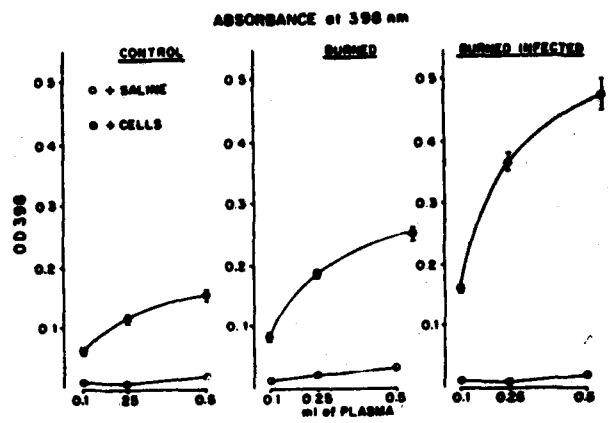


Figure 2. The interactions between plasma and cells in the generation of the biochemical indicators of infection.

Table 5. Localization of Biochemical Indicators

Indicator	Sample	Group		
		Control	Burned	Burned-Infected
Absorbance 398 nm	Whole blood	.096 ± .002	.123 ± .012	.617 ± .137
	Plasma	≤ 0	≤ 0	.012 ± .003
	Cells	.074 ± .019	.053 ± .010	.050 ± .012
	Plasma + cells	.060 ± .005	.047 ± .010	.324 ± .045
Fluorescence 280/340	Whole blood	395 ± 48	453 ± 21	1325 ± 65
	Plasma	1288 ± 75	1233 ± 29	3950 ± 129
	Cells	127 ± 25	103 ± 6	206 ± 118
	Plasma + cells	670 ± 53	613 ± 32	1588 ± 103
Fluorescence 355/420	Whole blood	21 ± 3	28 ± 2	143 ± 18
	Plasma	8 ± 1	10 ± 5	28 ± 5
	Cells	8 ± 1	8 ± 2	10 ± 2
	Plasma + cells	56 ± 6	56 ± 6	90 ± 6

n = 4; mean ± SD

Table 6. Effect of Cellular Elements of Blood on Generation of Biochemical Indicators of Infection

	OD 398	Fluorescence 280/340	Fluorescence 355/420
Whole blood	.306 ± .043	1198 ± 113	102 ± 19
Plasma + saline	.008 ± .003	2980 ± 191	25 ± 1
Plasma + cells	.199 ± .013	1706 ± 113	101 ± 15
Saline + cells	.025 ± .000	115 ± 12	13 ± 2
56° C plasma + cells	.216 ± .027	1680 ± 115	80 ± 14
PCA plasma + PCA cells	.124 ± .015	1780 ± 171	57 ± 5

n = 5; mean ± SEM

Ficoll-hypaque gradients were employed to ascertain what population of cells were requisite for the generation of the 398 and 355/420 factors. It would appear that the erythrocyte fraction (which also contains granulocytes in this preparation) is the required fraction (Table 8). Evidence that the granulocytes may not be the responsible cells is provided in

Table 7. Generation of Biochemical Indicators Dependent on Source of Plasma, Not of Cells

	OD 398	Fluorescence 280/340	Fluorescence 355/420
Plasma control + cells burned-infected	.047 ± .003	773 ± 40	28 ± 3
Plasma burned + cells burned-infected	.039 ± .006	720 ± 15	28 ± 3
Plasma burned-infected + cells burned-infected	.234 ± .027	2400 ± 177	111 ± 4
Plasma burned-infected + cells control	.255 ± .019	2338 ± 197	114 ± 11
Plasma burned-infected + cells burned	.239 ± .011	2375 ± 109	125 ± 11

n = 4; mean ± SEM

Table 8. Ficoll Gradient Separation of Cells Responsible for Generation of Biochemical Indicators of Infection

	OD 398	Fluorescence 280/340	Fluorescence 355/420
Plasma + saline	.039 ± .006	3112 ± 123	46 ± 3
Plasma + cells	.199 ± .023	1838 ± 131	116 ± 4
Plasma + supernatant	.011 ± .003	1200 ± 20	37 ± 2
Plasma + lymphocyte/ monocyte band	.036 ± .010	52 ± 2	49 ± 4
Plasma + RBC pellet	.338 ± .014	35 ± 14	108 ± 3

n = 4; mean ± SEM

Table 9, which indicates that plasma plus cells washed so as to remove the buffy coat are as effective, perhaps even more so, in promoting the formation of 398 and 355/420 factors. Additional studies need to be performed to purify and identify the substances in plasma and the moiety from cells which give rise to these biochemical indicators of infection.

Preliminary investigations indicate that when burned-infected plasma is treated with the proteolytic enzymes trypsin and pronase, the biochemical indicators are suppressed, leading one to believe that they are proteinaceous in nature.

Table 9. Buffy Coat Not Required for Generation of Biochemical Indicators

	OD 398	Fluorescence 280/340	Fluorescence 355/420
Whole blood	.548 ± .024	1163 ± 47	201 ± 12
Plasma + saline	.112 ± .022	3900 ± 71	78 ± 1
Plasma + cells with buffy coat	.324 ± .017	2138 ± 63	140 ± 5
Plasma + cells without buffy coat	.432 ± .012	1888 ± 66	201 ± 10

n = 4; mean ± SEM

PRESENTATIONS

Powanda MC: Partial characterization of biochemical indicators of infection in the burned rat. Seventeenth National Reticuloendothelial Society Meeting, Tampa, Florida, 4 December 1980.

Powanda MC: Indices of infection and/or inflammation in the burned and burned-infected rat. Sixty-fifth Annual Meeting, Federation of American Societies for Experimental Biology, Atlanta, Georgia, 15 April 1981.

Powanda MC: The role of leukocyte endogenous mediator (endogenous pyrogen) in inflammation. Symposium: The Roles of Copper and Other Essential Metals in Inflammatory Diseases, College of Pharmacy, University of Arkansas, Little Rock, Arkansas, 10 August 1981.

PUBLICATIONS

Powanda MC: Host metabolic alterations during inflammatory stress as related to nutritional status. *Am J Vet Res* 41:1905-1911, 1980.

Powanda MC, Dubois J, Villarreal Y, Walker HL, Pruitt BA Jr: Detection of potential biochemical indicators of infection in the burned rat. *J Lab Clin Med* 97:672-679, 1981.

Powanda MC, Moyer ED: Plasma proteins and wound healing. *Surg Gynecol Obstet* 153:749-755, 1981.

Powanda MC, Beisel WR: Hypothesis: Leukocyte endogenous mediator/endogenous pyrogen/lymphocyte activating factor modulates the development of nonspecific and specific immunity and affects nutritional status. *Am J Clin Nutr* (accepted for publication).

Powanda MC: Systemic alterations in metal metabolism during inflammation as part of the integrated response to inflammation. *In* Trace Elements in the Pathogenesis and Treatment of Inflammation. K.D. Rainsford, K. Brune and M.W. Whitehouse (eds.), Agents and Actions Supplements, Vol. 8, Birkhauser Verlag, Basel, 1981, pp 121-136.

Powanda MC, Moyer ED: Selected aspects of protein metabolism in relation to reticuloendothelial system, lymphocyte and fibroblast function. *In* The Reticuloendothelial System: A Comprehensive Treatise, Vol. 4 - Physiology of the Reticuloendothelial System. S.M. Reichard and J.P. Filkins (eds.), Plenum Press, New York. In press.

Powanda MC, Moyer ED: Plasma protein alterations during infection: Potential significance of these changes to host defense and repair systems. *In* Infection: The Physiologic and Metabolic Responses of the Host. M.C. Powanda and P.G. Canonico (eds.), Elsevier/North Holland Publishing Company, Amsterdam. In press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)436	
3. DATE PREV SUMMARY 80 10 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY ³ U	6. WORK SECURITY ⁴ U	7. REGRADING ⁵ NA	8. DD FORM INSTN ⁶ NL	9. SPECIFIC DATA ⁷ CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	10. LEVEL OF EFF A. WORK UNIT
10. NO./CODES ⁸		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
A. PRIMARY		61101A		3A161101A91C		00	
B. CONTRIBUTING						080	
C. CONTRIBUTING							
11. TITLE (Proceed with Security Classification Code) ⁹ (U) Assessment of Thyroid Hormone Kinetics in Thermally Injured Patients (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ¹⁰ 003500 Clinical Medicine							
13. START DATE 79 08		14. ESTIMATED COMPLETION DATE Cont		15. FUNDING AGENCY DA		16. PERFORMANCE METHOD C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				EXPIRATION:		FISCAL YEAR	
B. NUMBER: ¹¹				C. AMOUNT:		D. CUM. AMT.	
E. TYPE:				F. KIND OF AWARD:		G. FUNDING (in thousands)	
						1981 0.6 27	
						1982 0.4 25	
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: ¹² US Army Institute of Surgical Research				NAME: ¹³ US Army Institute of Surgical Research			
ADDRESS: ¹⁴ Ft Sam Houston, Texas 78234				ADDRESS: ¹⁵ Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr., COL, MC				NAME: ¹⁶ George M. Vaughan, MD, MAJ, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-5561			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
23. (U) Thyroxine; (U) l-triiodothyronine; (U) l-reverse-T ₃ ; (U) Kinetics; (U) Burn Patient							
24. (U) To assess metabolic clearance rate and production rate of thyroxine (T ₄), triiodothyronine (T ₃) in burned soldiers.							
25. (U) Six burn patients and two control unburned subjects were given an I.V. bolus of tracer T ₄ (¹³¹ I) and T ₃ (¹²⁵ I) with radioactive labels for single compartmental kinetic analysis.							
26. (U) 8010 - 8109. The results to date are reported for fiscal 1979-1980, and no further results have been obtained. However, we anticipate continuing these studies, utilizing an infusion technique that may obviate the need to use radioactive tracers.							

PROGRESS REPORT

**PROJECT NO. 31A61101A91C-00, IN-HOUSE LABORATORY
INDEPENDENT RESEARCH**

**REPORT TITLE: ASSESSMENT OF THYROID HORMONE KINETICS IN
THERMALLY INJURED PATIENTS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 August 1980 - 30 September 1981

**Richard A. Becker, M.D.
George M. Vaughan, M.D., MAJ, MC
Leonard G. Seraile, M.S.
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., COL, MC**

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY
INDEPENDENT RESEARCH

REPORT TITLE: ASSESSMENT OF THYROID HORMONE KINETICS IN
THERMALLY INJURED PATIENTS

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 August 1980 - 30 September 1981

Investigators: Richard A. Becker, M.D.
George M. Vaughan, M.D., Major, MC
Leonard G. Seraile, M.S.
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288 (R1)

Treatment of severe thermal injury is often seriously complicated by infection or coma, and low circulating levels of thyroid hormones have been associated with a predisposition to both in other kinds of patients. The mechanism by which burn injury produces low serum thyroid hormones (T_3 and T_4) in burn patients may provide a better understanding of this low T_3 syndrome. We are studying these hormones in order to determine if diminished production or accelerated disposal may account for the often profoundly low serum triiodothyronine (T_3) concentration seen in burn patients.

Thyroid hormone kinetics were assessed in six patients 18 to 45 years old and burned over 50% body surface and in two normal control subjects. Following bolus injection of isotopically labelled T_3 (^{125}I) and T_4 (^{131}I), the disappearance from serum of labelled hormone was followed over the next six days. A single compartmental model was used for analysis.

The half-life of T_4 was reduced to 2.2 days in burn patients from 4.8 days in controls. These data describe a high flow state for T_4 which has not been previously observed in any other non-thyroidal critical illness. The half-life of T_3 was reduced to 0.52 day after burns, as compared to 0.91 day in controls. A profound block of T_4 to T_3 conversion is apparent in burn patients, in that in spite of a threefold elevated clearance of T_4 and slightly elevated T_3 clearance and T_4 production, production of T_3 was half that in controls. These data are supportive of our earlier observation of a profound T_3 depletion state in critically ill burn patients due to decreased production and possibly accelerated clearance.

Thyroxine
Kinetics

Triiodothyronine
Burn patients

We plan to utilize a continuous infusion technique and include more controls, so that a more accurate estimation of the kinetics can be made. Measurement of reverse T_2 will be included, because other studies have indicated that it is markedly elevated in dying patients.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT COVERED PERIOD ³ DD-DRA&E(AR)696	
3. DATE PREV SUMMARY ⁴	4. KIND OF SUMMARY ⁵	6. DURN:TY SCTY ⁶	7. WORK SECURITY ⁷	8. REGRADING ⁸	9. DURN:TY SCTY ⁹	10. SPECIFIC DATA ¹⁰ CONTRACTOR ACCESS	11. LEVEL OF EFF ¹¹ A. WORK UNIT
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61101A	3A161101A91C	00	083				
13. PRIMARY							
14. CONTRIBUTING							
15. CONTRIBUTING							
16. TITLE (Provide with Security Classification Only) ¹⁶ (U) The Release of Mast Cell Mediators in the Thermally Injured Rat: A Preliminary Assessment for Study of Mast Cell Mediators in the Injured Soldier							
17. SCIENTIFIC AND TECHNOLOGICAL AREA ¹⁷ 003500 Clinical Medicine and 012600 Pharmacology							
18. START DATE	19. ESTIMATED COMPLETION DATE	20. FUNDING AGENCY		21. PERFORMANCE METHOD			
81 01	Cont	DA		C. In-House			
22. CONTRACT/GRANT		23. RESOURCES ESTIMATE		24. PROFESSIONAL MAN YRS		25. FUNDS (\$ in thousands)	
Not Applicable		PRESENT		1.0		12	
26. DATES/EFFECTIVE:		FISCAL YEAR		CURRENT			
27. NUMBER ²⁷		1981					
28. TYPE:		1982		0.8		30	
29. KIND OF AWARD:		30. CUM. AMT.					
31. RESPONSIBLE DSO ORGANIZATION		32. PERFORMING ORGANIZATION					
NAME ³¹ US Army Institute of Surgical Research		NAME ³² US Army Institute of Surgical Research					
ADDRESS ³¹ Ft Sam Houston, Texas 78234		ADDRESS ³² Ft Sam Houston, Texas 78234					
RESPONSIBLE INDIVIDUAL		PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)					
NAME: Basil A. Pruitt, Jr, COL, MC		NAME ³³ Roger W. Yurt, MAJ, MC					
TELEPHONE: 512-221-2720		TELEPHONE: 512-221-2968					
34. GENERAL USE		SOCIAL SECURITY ACCOUNT NUMBER:					
FOREIGN INTELLIGENCE NOT CONSIDERED		ASSOCIATE INVESTIGATORS					
		NAME:					
		NAME:		POC: DA			
35. REVISIONS (Provide SSAN for each revision) ³⁵							
(U) Rat Model; (U) Burns; (U) Mast Cells; (U) Histamine; (U) Mediators; (U) Leukocytes							
36. TECHNICAL OBJECTIVE, 37. APPROACH, 38. PROGRESS (Provide individual paragraphs identified by number. Provide text of each with security classification only.)							
<p>23. (U) The quantity of circulating mast cell mediators released during the early postburn period in the rat will be determined. The effect of such quantities of mediators on the immune response will be evaluated. This data will assist in formulation of pharmacologic approaches to modulation of edema and altered host defenses in the burned and injured soldier.</p> <p>24. (U) Rats will sustain thirty percent TBSA burns of either partial or full thickness depth. Sampling of blood via a subclavian catheter in the rat will be performed during the early post burn period. A radioenzymatic assay of histamine will be introduced to this laboratory to allow measurement of nanogram amounts of histamine in circulating blood. Additional mast cell mediators such as chemotactic factors will be measured by established methods and activated enzymes will be assayed by chromatographic separation of tritiated-DFP labeled material.</p> <p>25. (U) 8010 - 8109. Preliminary work indicates that a significant elevation of histamine occurs within one minute and five minutes after a 30% TBSA burn in the rat. This elevation persists to the end of the forty-five minute period studied. In contrast to initial experiments performed by the infusion of the selective mast cell activator, compound 48/80, where large amounts of histamine were released and peripheral neutrophil counts were depressed, significant leukocyte</p>							

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACRONYM	2. DATE OF SUMMARY	REPORT NUMBER	
				DA OG 1510	81 10 01	DD-DRAG(AR)436	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY DCTY	6. WORK SECURITY	7. RECLASS	8. EXPIRATION	9. SPECIFIC DATA CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
81 05 11	D. CHANGE	U	U	NA	NL	A. REPORT	
10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	61101A	3A161101A91C	00	083			
b. CONTRIBUTING							
c. CONTRIBUTING							
11. TITLE (Provide with security classification code) (U) The Release of Mast Cell Mediators in the Thermally Injured Rat: A Preliminary Assessment for Study of Mast Cell Mediators in the Injured Soldier							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS							
003500 Clinical Medicine and 012600 Pharmacology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
81 01		Cont		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
Not Applicable				PREVIOUS		b. FUNDS (in thousands)	
a. DATES/EFFECTIVE:				FISCAL YEAR		12	
b. NUMBER:				1981		1.0	
c. TYPE:				CURRENT		30	
d. KIND OF AWARD:				1982		0.8	
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: Roger W. Yurt, MAJ, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2968			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
23. KEYWORDS (Provide with security classification code)							
(U) Rat Model; (U) Burns; (U) Mast Cells; (U) Histamine; (U) Mediators; (U) Leukocytes							
24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with security classification code.)							
<p>changes have not been noted in the rat during the early post burn period. The present data suggest that the amount of histamine released during and after burn may be sufficient to modulate the immune response.</p>							

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1498, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

**PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT
RESEARCH**

**REPORT TITLE: THE RELEASE OF MAST CELL MEDIATORS IN THE THERMALLY
INJURED RAT: A PRELIMINARY ASSESSMENT FOR STUDY OF
MAST CELL MEDIATORS IN THE INJURED SOLDIER**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**Roger W. Yurt, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

**PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT
RESEARCH**

**REPORT TITLE: THE RELEASE OF MAST CELL MEDIATORS IN THE THERMALLY
INJURED RAT: A PRELIMINARY ASSESSMENT FOR STUDY OF
MAST CELL MEDIATORS IN THE INJURED SOLDIER**

**US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234**

Period covered in this report: 1 October 1980 - 30 September 1981

**Investigators: Roger W. Yurt, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

The quantity of histamine in the plasma of rats has been determined before and after thermal injury. Blood samples have been obtained through central venous cannulae which allows sequential sampling without perturbing the rat. These studies indicate that normal rat plasma histamine levels are lower than previously reported. Plasma histamine increases in proportion to extent of surface area burned and the kinetics of histamine appearance in the circulation differ between partial and full thickness injury. Evidence is presented to suggest that the kinetics of burn wound fluid accumulation differ between partial and full thickness injury. Mast cell mediators in addition may contribute to the accumulation of neutrophils in partial thickness burned skin.

**Rat Model
Burn Injury
Mast Cells**

**Histamine
Mediators
Leukocytes**

THE RELEASE OF MAST CELL MEDIATORS IN THE THERMALLY INJURED RAT

The mast cell with its array of chemical mediators has been held to regulate the tissue microenvironment (1). The isolation and characterization of multiple mast cell mediators, primarily from the rat peritoneal mast cell, has led to the concept of the mast cell being in part responsible for capillary dilatation and "leak", polymorphonuclear and eosinophil migration and deactivation, complement activation and modulation, platelet activation and anticoagulation. When the stimulus to mast cell activation is minimal, several processes act to limit the extent of mediator activity, such as hormonal regulation of cellular activation and the destruction of the mediators in the local environment. However when injury is extensive it has been suggested that mast cell mediators play a significant role in both the local and systemic response (2).

Previous reports suggest that mast cell release occurs after thermal injury based on measurement of plasma histamine. In these studies normal rat plasma histamine levels have been found to be 20 to 40 times that reported in human studies (3,4). In addition, standardized methods have not been applied to insure reproducible extent of injury with regard to depth and surface area injured. Initial investigation of mast cell mediator release in thermal injury has therefore been directed toward developing a standardized approach to measurement of mast cell mediators in a reproducible burn injury model. This report outlines the methods and results of study of plasma histamine after partial and full thickness injury in the rat. Evidence is presented to suggest that the mast cell may not influence the formation of edema in the burn wound although preliminary reports suggest that it regulates the ingress of polymorphonuclear leukocytes in the post injury period.

1. Austen KF: Homeostasis of effector systems which can also be recruited for immunologic reactions. *J Immunol* 121:793, 1978.

2. Yurt RW: Role of mast cells in trauma. In Dineen P (ed): *The Surgical Wound*. Philadelphia: Lea and Febiger. In press.

3. Beaven MA and Horakova Z: The enzymatic isotopic assay of histamine. *Handbook of Exp Pharmacology* 18:151, 1978.

4. Horakova Z and Beaven MA: Time course of histamine release and edema formation in the rat paw after thermal injury. *Eur J Pharmacology* 27:305, 1974.

METHODS AND MATERIALS

Male Sprague-Dawley rats (300-350g) were used in all experiments. In experiments requiring sequential blood sampling, the rats were cannulated according to the method of Harms and Ojeda (5) on the day prior to the study. Each rat was anesthetized with 0.1 ml Innovar by I.M. injection and Silastic brand silicone medical-grade tubing (0.05 cm ID, 0.09 cm OD) was passed from the external jugular vein into the superior vena cava. The proximal portion of the catheter was tunneled through the subcutaneous tissue and brought out a stab incision in the posterior aspect of the neck. The catheter was irrigated with saline and sealed between uses. One hundred to 250 microliter blood samples were drawn into 3.8% sodium citrate in a ratio of 10 to 1, respectively. The whole blood was placed in polypropylene tubes on ice and the plasma isolated by centrifugation at 1400 x g for 15 minutes and stored at minus 70°C in polypropylene tubes.

Rats were anesthetized with 25 mg/kg sodium pentobarbital and thermal injury was induced by the method of Walker-Mason in which the desired percentage burn was obtained by placing the rat in a mould such that a defined area of surface could be exposed to 95° water. Partial thickness injury was obtained by a two and one-half second exposure of the dorsal skin. Full thickness injuries occurred after 10 seconds of exposure of dorsal or two second exposure of ventral skin. Depth of injury was determined by histologic evaluation or clinical observation of the wound at two to three weeks post injury. Histologic evaluation of depth of injury was performed on hemotoxylin-eosin stained slides and Giemsa stained slides were evaluated for numbers of mast cells and polymorphonuclear cells. For evaluation of cellular response to injury, ten adjacent high power fields just above the skin - panniculus carnosus junction were evaluated for number of vessels, degranulated and normal mast cells, and polymorphonuclear leukocytes.

Skin water content was evaluated by immediately weighing tissue biopsies taken to the level of the fascia. Biopsies were then dried at 70°C until stable dry weights were obtained. Percent tissue water was calculated from the recorded wet and dry weights of the tissues. Histamine was measured by a radioenzymatic assay using histamine methyltransferase extracted from rat kidney (6).

5. Harms PG and Ojeda JR: A rapid and simple procedure for chronic cannulation of the rat jugular vein. *J Appl Physiol* 36:391, 1974.

6. Shaff RE and Beaven RE: Increased sensitivity of the enzymatic isotopic assay of histamine: Measurement of histamine in plasma and serum. *Anal Biochem* 94:645, 1979.

Data are expressed as means \pm standard error of the mean.

RESULTS

Rat plasma histamine before and after sham injury. Sham injured rats were handled the same as burn injured rats except for exposure to 95°C water. The combined data on the plasma of 55 rats studied at various times indicate that after anesthesia plasma histamine is 6.60 ± 0.40 ng/ml and that manipulation alone causes an early increase in plasma histamine to 22.75 ± 5.41 ng/ml at 1 minute after sham injury. Histamine levels drop to 11.54 ± 2.07 ng/ml by 2 minutes post sham and approximate presham levels by 5 minutes (Table 1). Sham injured rats who in addition received a 30 cc I.P. saline injection were found to have similar changes in plasma histamine.

Kinetics of systemic histamine release in 30% total body surface area full thickness burn. In rats studied from 0 to 45 minutes post burn, plasma histamine levels rose from 10.27 ± 1.90 at the 0 time to 88.01 ± 25.23 nanograms of histamine per ml at 30 minutes post burn (Figure 1). The sham injured animals had plasma histamine levels of $5.79 \pm .65$ at the 0 time point to a maximum of 8.76 ± 1.06 at the 45 minute time period. Clinical and histologic evaluation at 18 days post injury confirmed the injury as full thickness.

An additional study to evaluate the immediate post injury response showed that histamine was elevated to 37.4 ± 8.05 ng/ml ($n=5$) as early as 5 minutes post injury. Therefore, histamine levels were determined at 1, 2, 5 and 10 minutes post injury in a third experiment. Both sham and injured rats had elevated plasma histamine at 1 minute and both decreased at 2 and 5 minutes, however a second rise, consistent with previous findings, was noted at 10 minutes post burn that was not seen in the sham group (Figure 2). Clinical evaluation at 14 days post injury confirmed the full thickness nature of the burns.

Plasma histamine in partial thickness burn. In three rats sustaining partial thickness injury, plasma histamine levels ranged from $6.95 \pm .43$ at the 0 time point to a maximum of 18.6 ± 3.89 at 2 hours post injury. A gradual decrease in histamine levels occurred over the following two hour time period studied. Three sham burned rats had plasma histamine levels that were not significantly different from the burn injured rats (Figure 3). Histologic evaluation of biopsies taken 4 hours after injury confirmed that the burns were partial thickness. To further study the immediate response to burn injury in the partial thickness model, three groups of five rats each were studied. Two groups of rats sustained partial thickness

Table 1. Rat Plasma Histamine Before and After Sham Burn

Pre-Sham	Minutes After Sham					
	1	2	5	10	15	30 45
6.60(55)* +0.40	22.75(5) +5.41	11.54(9) +2.07	7.34(15) +1.05	7.18(19) +0.73	8.4(5) +1.97	6.97(10) +0.74 6.45(10) +1.01
I.P. Saline Group	19.28(5)** +1.96	13.99(5) +2.23	11.05(5) +2.87	7.82(5) +1.36		

*ng/ml(n)+S.E

**with 30ml saline injection I.P.

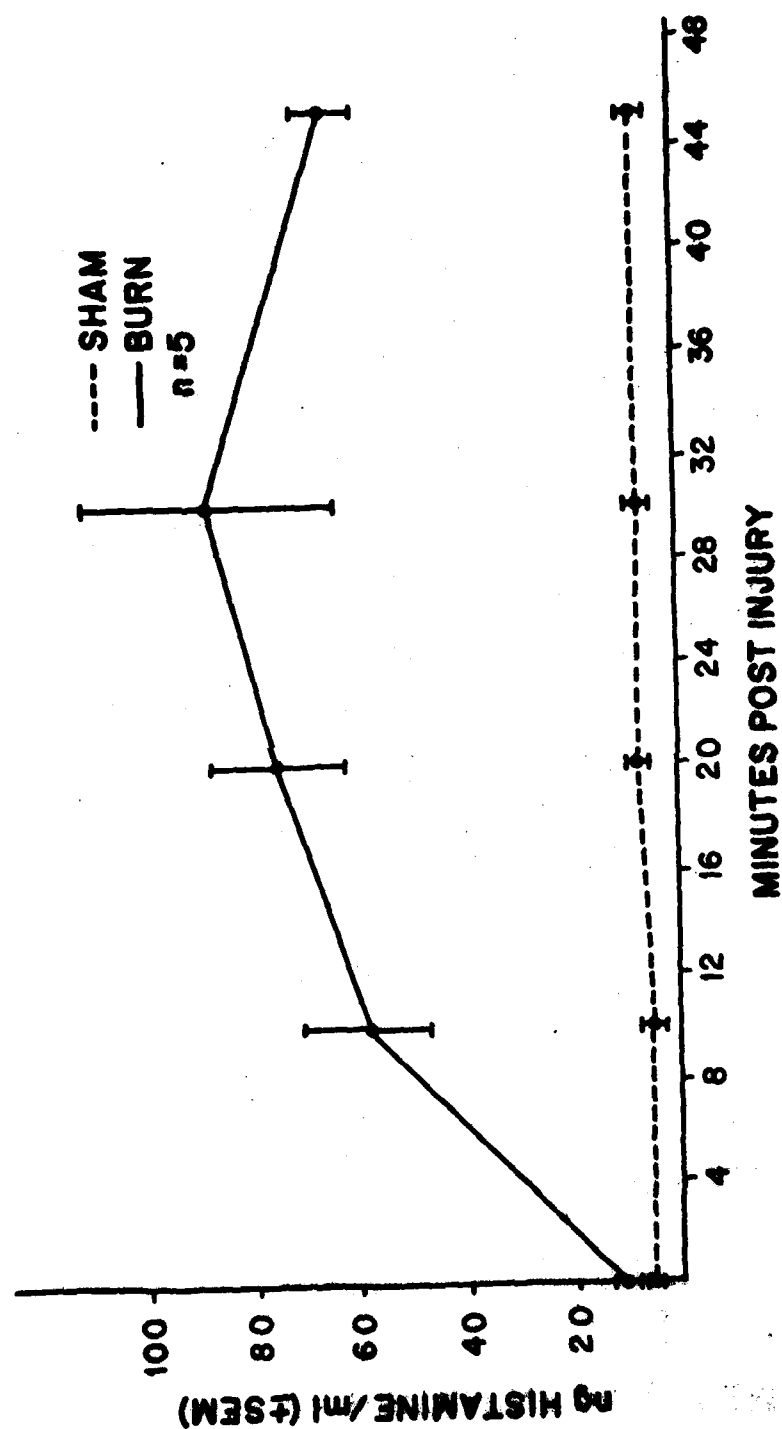


Fig. 1. Plasma histamine before and after 30% TBSA full thickness sham or burn injury.

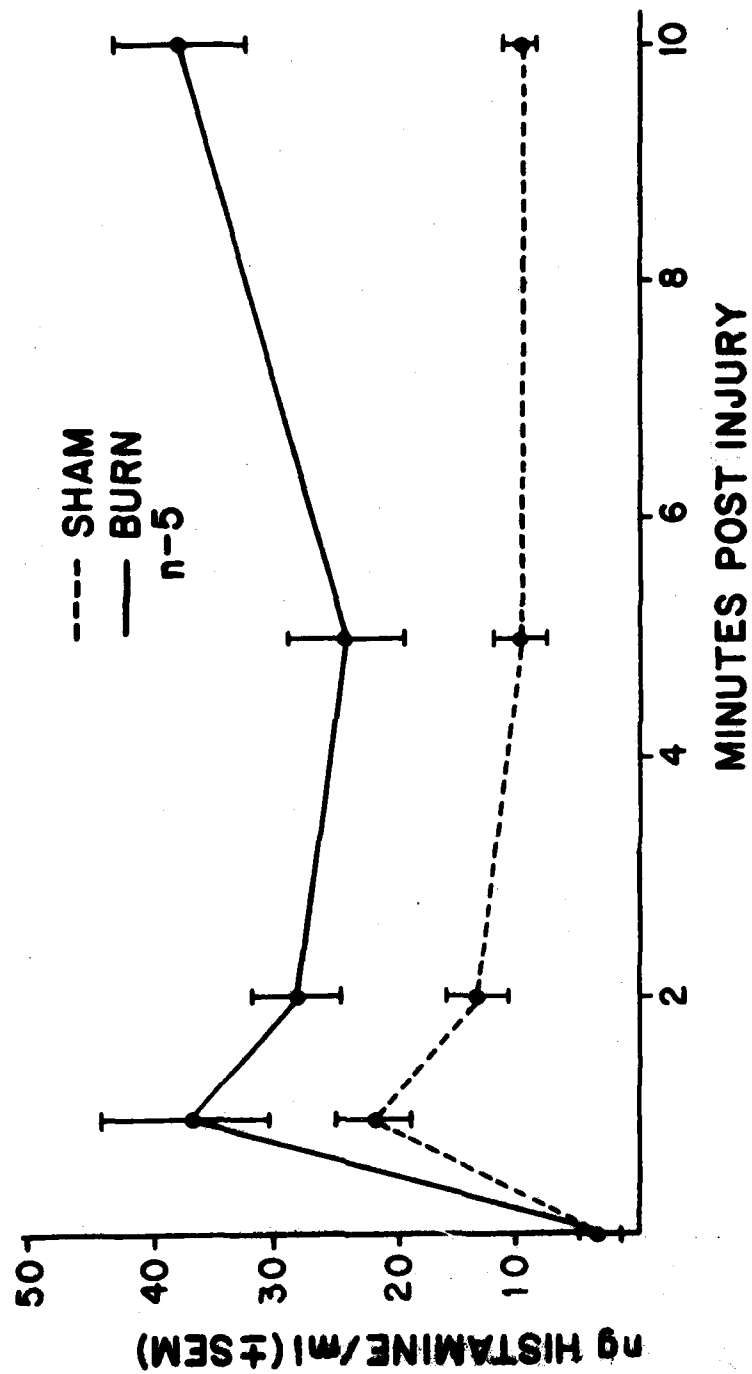


Fig. 2. Plasma histamine before and after 30% TBSA full thickness sham or burn injury.

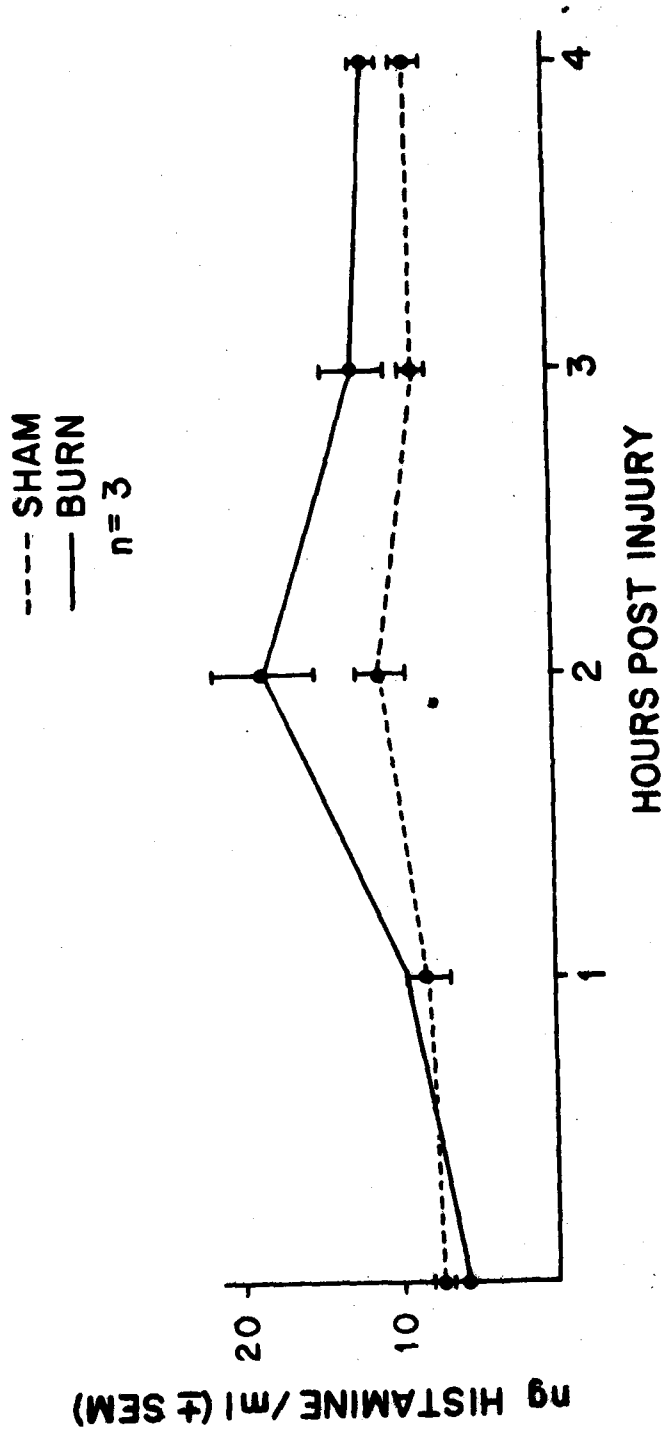


Fig. 3. Plasma histamine before and after 30% TBSA partial thickness sham or burn injury.

injury; in one group samples were obtained at 0, 1, 2, 5 and 10 minutes and in the additional burn group samples were obtained at 0, 2, 5, 10 and 15 minutes. The third group consisted of sham injured rats. Figure 4 shows that the sham burned rats had histamine levels ranging from 6.71 ± 1.37 at 0 time to 9.20 ± 3.38 nanograms per ml at 2 minutes. Peak levels of histamine occurred in the injured rats at 1 minute post injury with a mean of 49.88 ± 6.07 nanograms per ml. Histamine levels in the injured animals returned to levels twice normal at 15 minutes post injury.

To further evaluate the relationship between the extent of injury and histamine release, plasma histamine was measured after sham or 60% total body surface area partial thickness injury. Peak levels of histamine were seen to occur between one and two minutes post injury with a maximum of 81.03 nanograms per ml occurring at two minutes post burn. A smaller peak was noted in the sham group also occurring between one and two minutes with a mean of 19.28 ± 1.96 nanograms per ml occurring at one minute as compared to 0 time levels of 5.84 ± 1.72 nanograms per ml (Figure 5). Clinical evaluation at two and one-half weeks post injury showed that rats sustained a mean of 17.8 ± 1.11 percent full thickness and 42.2 percent partial thickness burns.

Plasma histamine levels after 30% total body surface area deep full thickness burn. Since there appeared to be a difference in the time-related increase in plasma histamine between partial and full thickness injury, systemic levels of histamine were determined between 5 and 45 minutes after deep full thickness burn. Preliminary experiments indicated that 12 second exposure of the dorsal surface of rats to 95°C water resulted in a deep burn including superficial portions of the panniculus carnosus. In the five rats sustaining deep injury under these conditions, the time course and quantity of histamine in the plasma (Figure 6) was not different from the more superficial full thickness injury. Sham injured animals had histamine levels between 3.06 ± 1.09 and 5.67 ± 1.56 nanograms per ml.

Kinetics of edema formation in 30% total body surface area partial and full thickness burn injury. To evaluate the time related development of edema in the early post burn period, 2 rats were sham burned and 8 sustained 30% BSA full thickness burns. Each of two rats were biopsied twice at 0 time (sham) or at 15, 30, 60 and 120 minutes. Unburned tissue contained 67% water and burned tissue rapidly increased in water content to 71% at 30 minutes post burn. This amount of water appeared to remain consistent over the subsequent 90 minute period studied.

In an additional study post injury water content of tissue was evaluated at an additional earlier time point of five minutes in

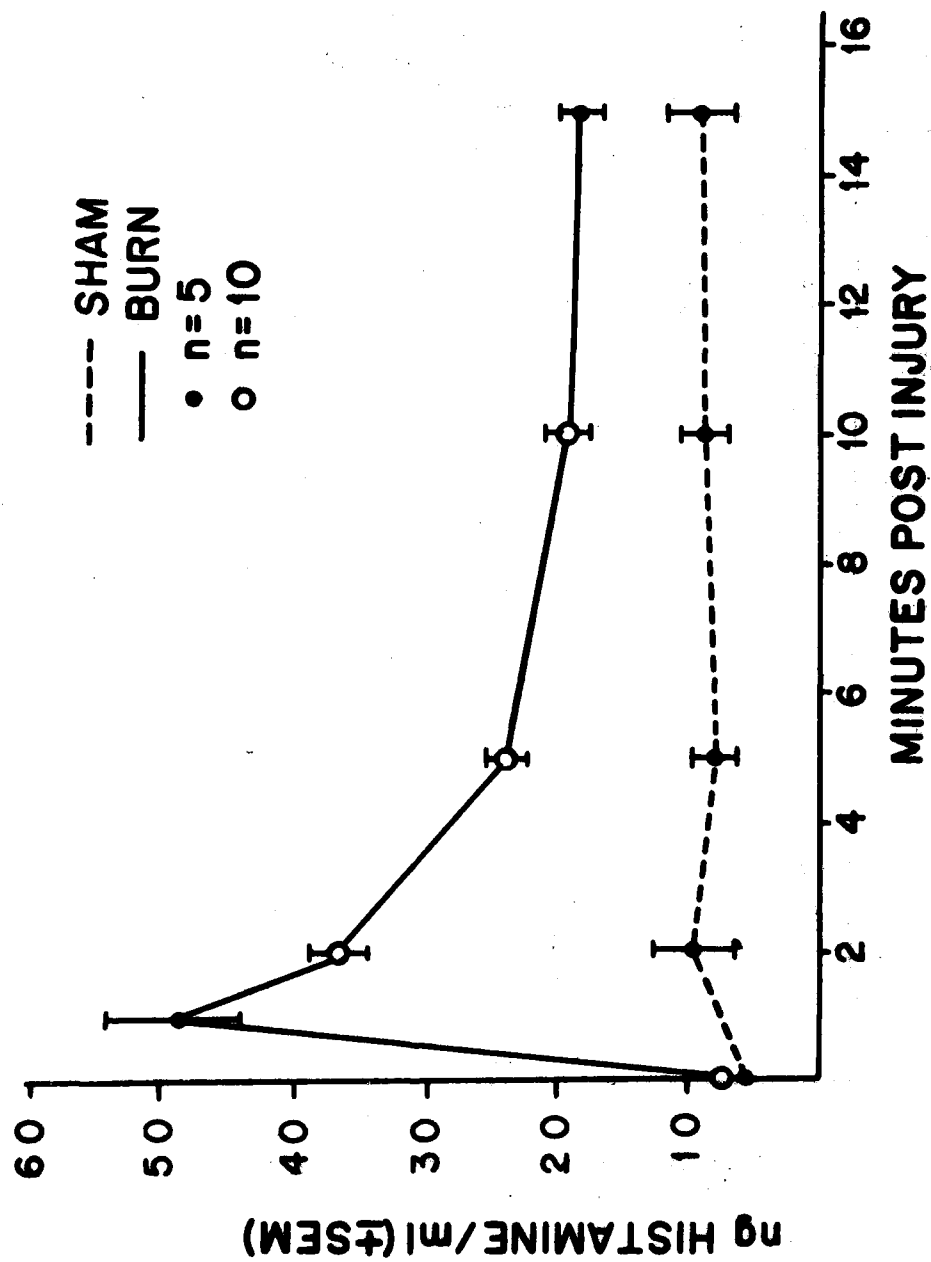


Fig. 4. Plasma histamine before and after 30% TBSA partial thickness sham or burn injury.

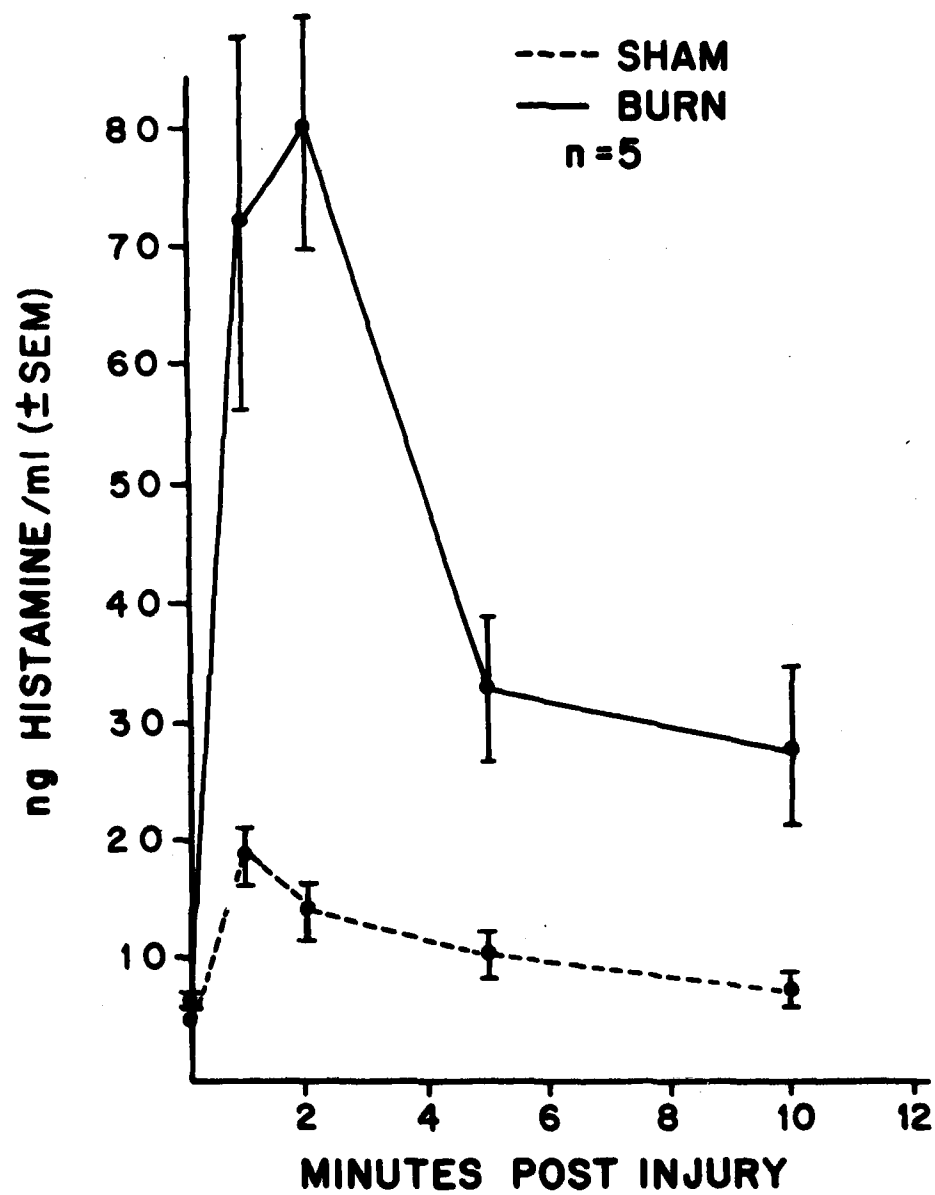


Fig. 5. Plasma histamine before and after 60% TBSA sham or burn injury.

rats sustaining sham, partial thickness, or full thickness burn injury (Figure 7). In both partial and full thickness injury tissue water rapidly increased and reached approximately the same levels by 10 to 20 minutes post injury; however more rapid accumulation of water appeared to occur in the full thickness injury by the five minute time period. There was no statistical difference in skin water between 20 and 45 minutes.

Effects of mast cell depletion on edema formation, histamine release, and neutrophil margination and infiltration. To determine the effect of mast cell depletion, four rats were treated with Polymyxin B and four with saline prior to injury. Half of each group was then either sham burned or burned and plasma histamine was determined at 0, 5, 15 and 30 minutes post injury. Biopsies were taken one hour post injury and percent skin water determined on the basis of two biopsies per rat. In addition, biopsies were taken for histologic evaluation. Sham burned rats (Table 2) had low levels of plasma histamine at all time points; however Polymyxin B pretreated rats had somewhat higher plasma histamine. Polymyxin B pretreated burned rats had slight elevations of histamine at 5 minutes post injury however not to the extent seen in the saline pretreated rats that had levels of 33.01 nanograms per ml at 30 minutes post injury. The percentage of water in the skin biopsies was not different between the saline and Polymyxin pretreated sham injured rats nor was it different between rats pretreated with saline or Polymyxin B prior to burn. There was a substantial decrease in numbers of mast cells in Polymyxin B pretreated sham and burn injured animals. In saline pretreated animals the burned tissue contained 5.32% degranulated mast cells whereas burned tissue contained 20.09% degranulated cells. Percent degranulation of Polymyxin B treated animals is based on very few mast cells in the total sample. Accumulation of neutrophils in the tissue biopsies revealed a higher number of neutrophils per vessel in the saline pretreated burned rat as compared to others; however the groups were too small to show statistical difference. Since full thickness injury appeared to cause impairment of blood flow to the skin, the effects of mast cell depletion were then studied in partial thickness injury. Table 3 shows the findings at 4 hours post injury in rats pretreated with saline followed by sham or burn injury and in an additional group of Polymyxin B pretreated animals who sustained 30% total body surface burn. Burn injury led to increased percent water in the tissue at four hours however there was no difference between saline and Polymyxin B pretreatment of burns with regard to tissue water content. There was a striking difference between the number of neutrophils infiltrating the wound when saline pretreated burn rats are compared to saline pretreatment with sham injury or to Polymyxin B pretreatment with burn injury. The effectiveness of Polymyxin B pretreatment in depletion of mast cells was again seen where only 0.09 cells per

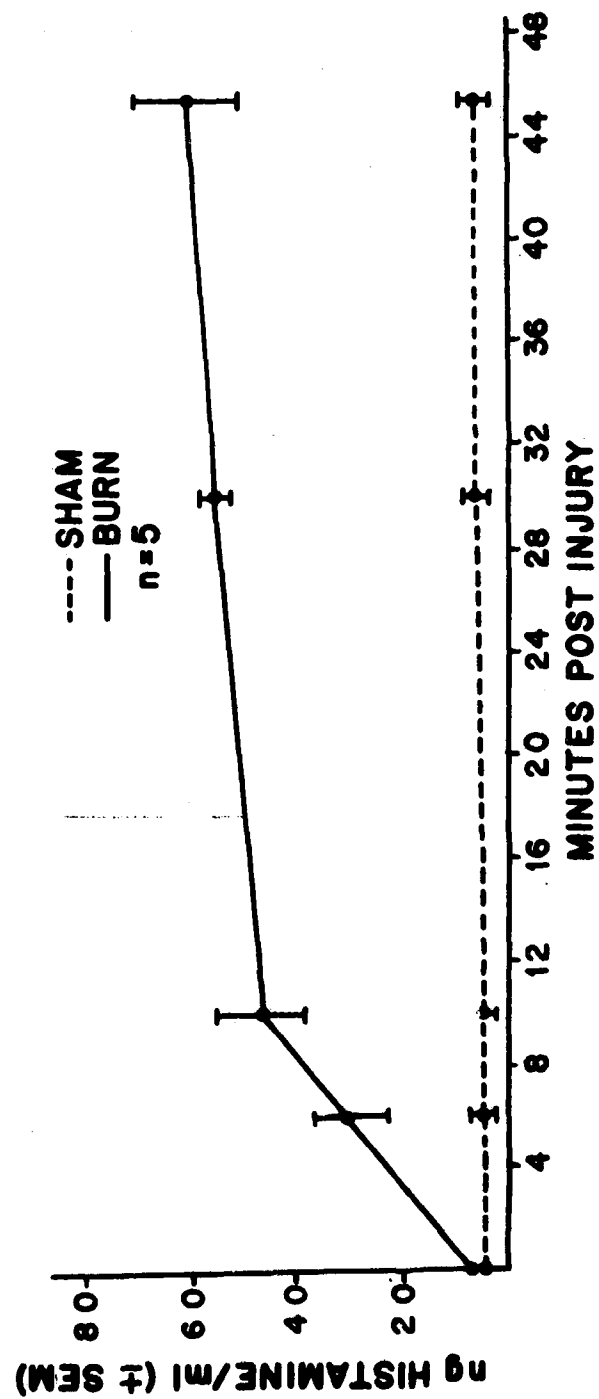


Fig 6. Plasma histamine before and after 30% TBSA deep full thickness sham or burn injury.

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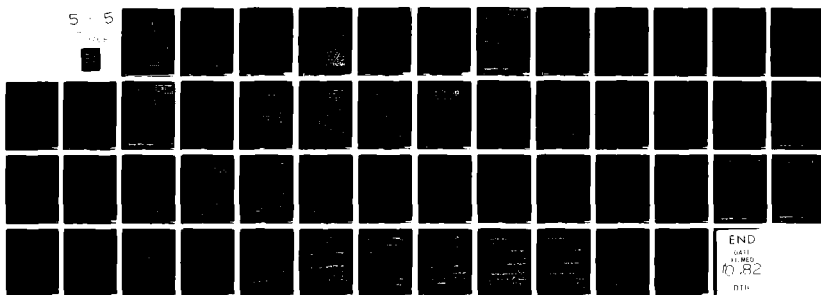
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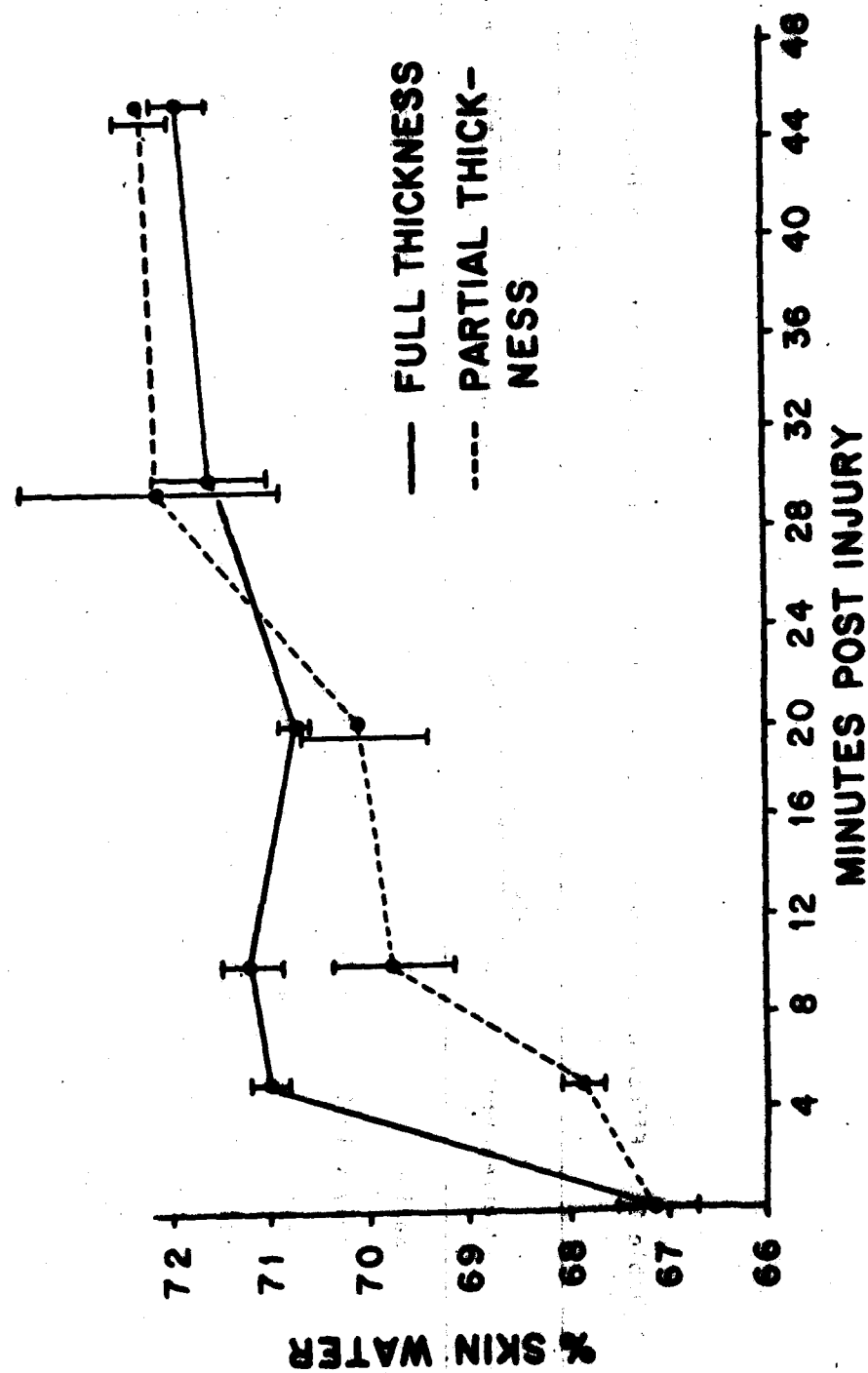


Fig 7. % skin water after 30% TBSA partial and full thickness burn.

Table 2. Effect of Polymyxin B Pretreatment on Sham or Full Thickness Burn (Injured Rats)

Injury	Pretreatment	Histamine (ng/ml plasma)				% Water	Mast Cells*		# PMN**
		0	5	15	30		No.	***	
Sham	Saline	1.74	1.60	0.00	0.57	67.85	2.02	5.36	0.135
Sham	Polymyxin B	6.72	6.01	11.02	9.47	67.84	0.20	73.45	0.15
Burn	Saline	3.01	16.68	32.75	33.01	72.50	1.46	20.09	0.615
Burn	Polymyxin B	6.72	12.93	11.20	12.93	73.65	0.19	52.75	0.45

*Number of mast cells/vessel

**Number of polymorphonuclear leukocytes/vessel

***Percent degranulation

Table 3. Effect of Polymyxin B Pretreatment on Partial Thickness Burn Injured Rats

Injury	Pretreatment	% Water	# PMN***	Mast Cells***	
				No.	% Degranulated
Sham	Saline*	65.92 ± .51	0.08	1.43	17.2
Burn	Saline*	72.64 ± .85	5.17	1.57	61.1
Burn	Polymyxin B**	72.16 ± .62	0.65	0.09	46.7

*n=4
*n=3
***cells/vessel

vessel were found in the Polymyxin B pretreated animals as compared 1.57 and 1.53 mast cells per vessel in saline pretreated burn and sham animals respectively. A larger number of mast cells were seen to be degranulated in the saline pretreated burned animal as compared to the sham animal. In an additional study of the effects of Polymyxin B pretreatment in which all rats sustained 30% TBSA partial thickness burns, 5 rats were pretreated with saline and five rats pretreated with Polymyxin B (Table 4). There appeared to be no difference in percent tissue water between the groups at four hours; however there again were less neutrophils per vessel in the Polymyxin B pretreated rats. The mast cells appeared to be depleted although not to the same extent as in the previous experiment.

DISCUSSION

Rat plasma histamine levels have been found to be higher than those reported for humans (6). However in the absence of traumatic blood sampling, levels reported here are one-half (3) to one-eighth (4) of those previously reported in rats. In addition the use of indwelling catheters has allowed for evaluation of nanogram changes in plasma histamine level that can be attributed to burn injury. Thirty percent total body surface area full thickness burns lead to rapid increases in histamine occurring as early as one minute post injury. After a transient decrease, these levels continue to rise and appear to plateau at levels of six to eight times normal at the latest time period studied of 45 minutes. In contrast partial thickness injury whether it be 30% or 60% leads to acute changes that are proportional to surface area burned but do not lead to the secondary sustained elevation as seen in full thickness injury.

Edema formation appears to occur quite rapidly in both partial and full thickness burns and temporally is related to early systemic increases in histamine. On the other hand, evidence has been presented that histologically confirmed depletion of mast cells by Polymyxin B does not influence edema formation as measured at one and four hours post burn. It is of interest to note that there appears to be a difference in the rapidity of edema formation at the earliest time studied of five minutes with partial thickness injury lagging behind full thickness. Such findings may represent a disproportion between fluid influx into and efflux from the wound. Full thickness injury with early and progressive diminution of blood flow and increased osmolarity due to tissue destruction leads to trapping of fluid; and therefore more rapid accumulation of fluid whereas partial thickness injury may permit a more nearly equal flux in both directions resulting in slower fluid accumulation. The kinetics of histamine release may merely reflect this phenomenon and the progressive nature of the full thickness injury. Even though preformed mast cell mediators may have little effect on edema formation, these

Table 4. Effect of Polymyxin B Pretreatment on Partial Thickness Burn Injured Rats

Injury	Pretreatment	% Water	# PMN**	Mast Cells**	
				No.	% Degranulated
Burn	Saline*	71.41 \pm .35	4.87 \pm .58	1.52 \pm .15	63.1 \pm 9.2
Burn	Polymyxin B*	70.60 \pm .18	1.13 \pm .16	0.28 \pm .06	75.0 \pm 6.6

*n=5

**Number of cells/vessel

preliminary studies suggest that the mast cell may in part mediate the acute polymorphonuclear leukocyte response to burn injury.

PUBLICATIONS/PRESENTATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACRONYM	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL	
				DA 9G 6979	81 10 01	DD-DRAE(AR)696	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISSEM INSTR	9. LEVEL OF EFF	
81 10 01	K. COMP	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO A. WORK UNIT	
10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
A. PRIMARY	61101A	3A161101A91C	00	085			
B. CONTRIBUTING							
C. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) (U) Mitochondrial Oxidative Function In The Burn Wound and The Effect of Resuscitation In Burned Soldiers (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
78 09		81 09		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
Not Applicable				PRECEDES		b. FUNDS (in thousands)	
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b. NUMBER:				1981		23	
c. TYPE:				CURRENT		0.0	
d. KIND OF AWARD:				1982		00	
20. RESPONDER'S ORG ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Precede with U.S. Acronym if applicable)			
NAME: Basil A. Pruitt, Jr, MD, COL, MC				NAME: Cleon W. Goodwin, Jr., MD, FACS			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-5712			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
23. REVERSE (Precede each with Security Classification Code)							
(U) Metabolism; (U) ATP; (U) Oxygen; (U) Cytochromes; (U) Goats							
24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Precede individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To define pathologic alterations in cellular function produced by thermal injury and to assess efficacy of treatment in reversing the process in burned soldiers.</p> <p>24. (U) Initial studies will utilize the burned rat as a model for human burns. Following specific interval of time, sample of granulation tissue from burned areas will be sampled for separation of its subcellular components. Mitochondrial oxidative phosphorylation will be assayed by appropriate techniques, oxygen uptake by polarographic electrode, cytochrome content and activity by the double beam dual wave length spectrophotometry, and calcium transport by its reaction to murexide. After these baseline data are obtained, the effects of various resuscitation formulae in improving tissue perfusion will be assessed by changes in cellular functions described above.</p> <p>25. (U) 8010 - 8109. The mechanism of increased oxygen utilization accompanying the onset of postinjury hypermetabolism was studied in liver mitochondria obtained from control (C), partially starved (PS), and 50% TBS burned (B) rats (an established hypermetabolic animal model, $VO_2 \uparrow$ 30 to 40%). Mitochondrial oxygen utilization (state 3) increased significantly ($p < .01$) in the injured animals: (C) 38.56 vs (PS) 39.75 vs (B) 50.38 nmoles O_2/min/mg protein. Oxidation was not uncoupled from phosphorylation. This augmented energy efficient oxygen uptake by mitochondria explains in large measure the increased total body oxygen consumption associated with postinjury hypermetabolism. This phase of the study is now complete.</p>							

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MITOCHONDRIAL OXIDATIVE FUNCTION IN THE BURN
WOUND AND THE EFFECT OF RESUSCITATION IN BURNED
SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1980 - 30 September 1981

Investigators:

Cleon W. Goodwin, M.D.
Joseph Whitson, Sp5
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDON 286 (R)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MITOCHONDRIAL OXIDATIVE FUNCTION IN THE BURN
WOUND AND THE EFFECT OF RESUSCITATION IN BURNED
SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1980 - 30 September 1981

Investigators: Cleon W. Goodwin, M.D.
Joseph Whitson, SP5
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

The mechanism of increased oxygen utilization accompanying the onset of postinjury hypermetabolism in liver was studied in mitochondria obtained from control, partially starved, and 50% total body surface burned rats (an established hypermetabolic animal model- $\dot{V}O_2$ 30 to 40%+). Resting oxygen uptake (in the presence of substrate, state 4), maximal oxygen uptake (following addition of ADP, state 3), respiratory control ratios (RCR, and index of mitochondrial integrity) and phosphorylation rates were calculated. Mitochondrial oxygen utilization increased significantly in the injured animal and was paralleled by an increase in phosphorylation of ADP to ATP. These changes could not be explained by weight loss. Oxidation was not uncoupled from phosphorylation. This augmented energy efficient oxygen uptake and energy production of mitochondria explains in part the increased total body oxygen consumption associated with postinjury hypermetabolism.

Oxidative phosphorylation
Coupling
Mitochondria
Oxygen uptake

MITOCHONDRIAL OXIDATIVE FUNCTION IN THE BURN WOUND AND THE EFFECT OF RESUSCITATION IN BURNED SOLDIERS

The stress of large thermal injuries initiates alterations of body homeostasis which are primarily catabolic in nature and which manifest as increased metabolic rate, erosion of body mass, loss of nitrogen in the urine, and abnormalities of carbohydrate metabolism. Following resuscitation, metabolic rate rises and is accompanied by an increase in total body oxygen consumption, which is proportional to the extent of injury. This increase varies with time postinjury and may reach levels exceeding twice that of uninjured patients before declining in a curvilinear fashion as the underlying pathologic process resolves (1-3). Total body blood flow (cardiac index) increases in parallel with the rise in oxygen consumption, approaches a plateau which may exceed 2-3 times that of uninjured individuals, and declines as the patient recovers (1,2). Regional blood flow to the viscera increases in a similar fashion, although its fraction of the total flow remains unchanged or only slightly increased (4). Similarly, viscera oxygen utilization increases proportionately, thus maintaining an unchanged or slightly elevated fraction of total body oxygen consumption. During the hypermetabolic phase of burn injury, the liver is the most metabolically active organ, and this level of metabolic work is reflected by its increased oxygen utilization. Oxygen utilization by the liver at the subcellular level occurs predominantly in the mitochondria. To assess any alterations of oxygen utilization induced by the hypermetabolic response following thermal injury, mitochondrial function was assayed in a hypermetabolic femoral injury animal model.

METHODS

Animal Preparation

Male Holzman rats (475 to 500 grams body weight) were placed in single cages and allowed to acclimate for two weeks in a light tight environmental room. During this stabilization, the rats were entrained to light on a 0600

1. Gump FE, Kinney JM: Energy balance and weight loss in burned patients. Arch Surg 103: 442-48, 1971
2. Gump FE, Price JB, Jr., Kinney JM: Blood flow and oxygen consumption in patients with severe burns. Surg Gynecol Obstet 130: 23-8, 1970
3. Wilmore DW, Long JM, Mason AD, Jr., Skreen RW, Pruitt BA, Jr.: Catecholamines: Mediator of the hypermetabolic response to thermal injury. Ann Surg 180: 653-69, 1974
4. Wilmore DW, Goodwin CW, Aulick LH, Powanda MC, Mason AD, Jr., Pruitt BA, Jr.: Effect of injury and infection on visceral metabolism and circulation. Ann Surg 192: 491-504, 1980

to 2000 hours on - 2000 to 0600 hours off cycle. Temperature was controlled at $27 \pm 2^\circ\text{C}$. Animals were fed a laboratory chow diet which was maintained in excess in all cages until study period began. Animals were then randomly assigned to three groups: CONTROL animals were allowed to eat ad libitum for an additional twelve days. BURNED animals were subjected to a standardized 30 percent total body surface scald burn as described by Herndon et al (5). Following the injury, the animals were allowed to recover and consumed an ad libitum diet for twelve days. PARTIALLY STARVED animals were fed a restricted quantity of chow diet so as to produce a weight loss identical to the burned animal to which it had been paired. This restricted diet in the PARTIALLY STARVED animals continued for twelve days. At the end of the twelve days each animal was sacrificed following a 16 hour fast.

Isolation of Mitochondria

Following its removal, liver (approximately 5 grams) was placed in a 0°C incubation solution and chopped into small pieces to facilitate rapid cooling. Mitochondria were isolated in a medium consisting of 0.225 M mannitol, 0.075 M sucrose, 100 μM EGTA, and a final pH of 7.4. The mitochondria were gently homogenized by a motor-driven Teflon pestle in a glass homogenizer. The resulting suspension was centrifuged at 600 X g to remove residue, and the mitochondria were washed four times and recovered at 8000 X g. Washed mitochondria were suspended in an EGTA-free medium at 20-30 mg protein per ml.

Mitochondrial Assays

All measurements were carried out in a medium containing 0.225 M mannitol, 0.075 M sucrose, 15 mM TRIS, 10 mM KH_2PO_4 , and a final pH of 7.4. Oxygen uptake was measured polarographically by a Clarke O_2 electrode with mitochondria respiring in state 4 (excess substrate) and state 3 (excess substrate and ADP) (6). Respiratory control ratios (RCR) were calculated as the ratio of state 3 to state 4 rates. ADP/O ratios were calculated from the measured O_2 consumption (O_2 capacity of medium-240 nanomoles/ml) with 500 μM ADP as the phosphate acceptor. Protein concentrations of the mitochondrial samples were determined by a modification of the biuret reaction (7).

Statistics

The data were analyzed by analysis of variance. A probability of less than 0.05 was used to judge significant differences between treatment groups.

5. Herndon DN, Wilmore DW, Mason AD, Jr.: Development and analysis of a small animal model simulating the human postburn hypermetabolic response. *J Surg Res* 25: 394-403, 1978
6. Chance B, Williams GR: The respiratory chain and oxidative phosphorylation. *Adv Enzymol* 17: 65-134, 1956
7. Cornell AG, Bardawill CJ, David MM: Determination of serum proteins by means of the biuret reaction. *J Biol Chem* 177: 751-66, 1949

RESULTS

Utilizing either succinate or glutamate plus malate as substrates, mitochondrial oxygen uptake increased significantly in the injured animal when compared to control (Tables 1 and 2). However, partial starvation did not affect mitochondrial oxygen utilization. The phosphorylation rate, the product of ADP/O ratio and the state 3 oxygen utilization rate, also increased significantly in the injured animals but not in the partially starved animals. There were no differences among the respiratory control ratios of all three study groups. The uninjured control animals continued to gain weight slowly over the twelve day study period while the partially starved and injured animals lost approximately 10 percent of their initial body weight over the twelve day study interval.

DISCUSSION

Substrate entry into the mitochondrial electron transport chain and subsequent energy production occurs at two primary sites. Reducing equivalents produced by substrates typified by glutamate and malate enter the electron transport chain by way of nicotinamide adenine dinucleotide (NAD). The other major entry site, utilized by substrates typified by succinate, takes place at the flavin adenine dinucleotide (FAD). Utilizing both sets of substrates in the above experiments, increased mitochondrial oxygen utilization, and by inference, increased energy production, occurred during the hypermetabolic phase of burn injury in the experimental rat model. Mitochondrial oxygen utilization was unaffected by weight loss alone but increased in parallel with ADP phosphorylation in the injured animals. Since the respiratory control ratios, an index of efficiency of oxygen utilization remained unchanged after injury and after starvation, there was no evidence of an uncoupling effect induced by either of these physiological stresses. Oxygen utilization remained efficient in the hypermetabolic animal. This significantly augmented energy efficient mitochondrial oxygen uptake in burned animals correlates well with the increased total body oxygen consumption characteristic of postinjury hypermetabolism. Total body oxygen uptake by this hypermetabolic animal model typically increases 30-40% and this compares with the approximately 30-40% increase in mitochondrial oxygen utilization. The initial stimulus to this increased oxygen uptake and hypermetabolism remains undefined, but a likely candidate is the circulating catecholamines or glucagon known to be increased during the hypermetabolic phase of burn injury (3,8).

8. Harrison TS, Seaton JF, Feller I: Relationship of increased oxygen consumption to catecholamine excretion in thermal burns. *Ann Surg* 165: 169-72, 1967

PUBLICATIONS/PRESENTATIONS - None

Table 1. Mitochondrial Function: Glutamate and Malate as Substrate

	CONTROL Mean (S.D.)	PARTIALLY STARVED Mean (S.D.)	BURNED Mean (S.D.)
State 3 (nmoles O ₂ · min ⁻¹) <u>(mg protein)</u>	38.56 (⁺ 3.14)	39.75 (⁺ 4.37)	50.38* (⁺ 2.52)
RCR (State 3/State 4)	6.64 (⁺ 0.62)	6.86 (⁺ 0.89)	7.03 (⁺ 0.56)
Phosphorylation rate (nmoles ADP · min ⁻¹) <u>(mg protein)</u>	110.0 (⁺ 5.4)	110.9 (⁺ 13.3)	143.6* (⁺ 10.1)
Weight Change (%)	+4%	-11%	-12%

*Control vs burn, $p < .01$ by ANOVA; n=10 in each group

Table 2. Mitochondrial Function: Succinate as Substrate

	CONTROL Mean (S.D.)	PARTIALLY STARVED Mean (S.D.)	BURNED Mean (S.D.)
State 3 (nmoles O ₂ · min ⁻¹) <u>(mg protein)</u>	46.52 (⁺ 8.23)	47.76 (⁺ 3.75)	62.95* (⁺ 10.68)
RCR (State 3/State 4)	5.30 (⁺ 0.93)	5.33 (⁺ 0.42)	5.17 (⁺ 0.72)
Phosphorylation Rate (nmoles ADP · min ⁻¹) <u>(mg protein)</u>	82.35 (⁺ 6.40)	84.53 (⁺ 6.23)	110.16* (⁺ 11.47)

*Control vs burn, $p < .01$; $n=12$ for each treatment group

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACRONYM	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL	
				DA OG 5028	81 10 01	DD-DRA&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. RESEARCHING	8. DESIGN INSTR	9. SPECIFIC DATA - CONTRACTOR ACCESS	
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		61101A		3A161101A91C		00 087	
11. PRIMARY							
12. CONTRIBUTING							
13. CONTRIBUTING							
14. TITLE (Precede with Security Classification Code)							
(U) Role of Lipid Metabolism in Burn Injury (44)							
15. SCIENTIFIC AND TECHNOLOGICAL AREA							
003500 Clinical Medicine and 012900 Physiology							
16. START DATE		17. ESTIMATED COMPLETION DATE		18. FUNDING AGENCY		19. PERFORMANCE METHOD	
80 02		Cont		DA		C. In-House	
20. CONTRACT/GRANT				21. RESOURCES ESTIMATE		22. PROFESSIONAL MAN YRS	
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B. NUMBER:				1982		45	
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D. KIND OF AWARD:							
E. CUM. AMT.							
23. RESPONSIBLE DOD ORGANIZATION				24. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Precede with U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: David R. Strome, CPT, MSC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2968			
25. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
26. KEYWORDS (Precede each with Security Classification Code)							
(U) Lipid Metabolism; (U) Fatty Acid Oxidation; (U) Mass Spectroscopy; (U) Burn Injury; (U) Mitochondrial; (U) Gluconeogenesis; (U) Lab Animal							

23. (U) To evaluate in an animal model the changes in lipid metabolism which have been observed following thermal injury in burned soldiers and to assess the effectiveness of conventional nutritional support in the presence of these alterations.

24. (U) The isolated adipocyte is being used to determine the metabolic response of adipose tissue to various hormonal alterations associated with thermal injury. Changes in tissue lipid composition and metabolic pathways are being investigated using gas chromatography-mass spectroscopy.

25. (U) 8010 - 8109. The initial experiments have demonstrated a significant decrease in the ability of epinephrine to stimulate the breakdown of triglycerides in adipocytes from burned animals. This response is not present on the first day post-burn, but is apparent by the fifth day and continues unabated through the twentieth day, which was the last experiment day. The basal rates of triglyceride breakdown were not different between burned and unburned animals. With respect to lipid composition, techniques have been established and confirmed for continuing this part of the investigation.

ANNUAL PROGRESS REPORT

**PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY
INDEPENDENT RESEARCH**

**REPORT TITLE: THE ROLE OF LIPID METABOLISM IN BURN INJURY:
ALTERATIONS IN ADIPOCYTE RESPONSIVENESS TO
HORMONAL STIMULATION**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**David R. Strome, Ph.D., Captain, MSC
Cleon W. Goodwin, Jr., M.D.
Arthur D. Mason, Jr., M.D.**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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**REPORT TITLE: THE ROLE OF LIPID METABOLISM IN BURN INJURY:
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To study the effects of severe burn injury on lipid metabolism, we compared rates of lipolysis in isolated adipocytes obtained from control rats and from rats 1, 5, 10, 15 and 20 days following a 60% total body surface burn. Lipolysis was measured as glycerol production in basal and stimulated (10^{-5} M epinephrine) conditions. Adipocyte responsiveness to hormonal stimulation was determined to be the difference between stimulated and basal lipolytic rates. Basal glycerol production was indistinguishable in cells from burned and control rats. However, adipocytes from burned animals showed a decrease in responsiveness to epinephrine as early as the fifth postburn day. This depressed response continued throughout the 20 days of the experiment. It is concluded that burn injury results in major alterations at the cellular level in adipose tissue. One of these alterations is decreased lipolytic responsiveness to catecholamine stimulation. This may be due to changes in either the cell membrane or the intracellular enzyme pathways in response to the hormonal environment.

**Adipocytes
Glycerol
Epinephrine**

THE ROLE OF LIPID METABOLISM IN BURN INJURY: ALTERATIONS IN ADIPOCYTE RESPONSIVENESS TO HORMONAL STIMULATION

Severe injury is characterized in part by a hypermetabolic condition which includes increased mobilization of body fat (1, 2). Accompanying this increased mobilization of lipids are increased serum fatty acids (3), increased glycerol turnover (4), increased clearance rate for infused triglycerides (5) and depletion of body fat stores (6, 7).

The mobilization of lipid is primarily under hormonal control, mainly circulating epinephrine, norepinephrine, insulin and glucagon. During the hypermetabolic period, circulating catecholamines are chronically elevated (8, 9, 10); whereas, insulin and glucagon tend toward normal after an early change (11, 12, 13). Because of the strong lipolytic effects of epinephrine, it is possible that the observed increase in lipid breakdown may be directly related to the elevated hormonal levels.

1. Elwyn DH: Nutritional requirements of adult surgical patients. *Critical Care Medicine* 8:9-20, 1980.
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7. Davies JWL, and Fell GS: Tissue catabolism in patients with burns. *Clinica Chimica Acta* 51:83-92, 1974.
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9. Aikawa N, Caulfield JB, Thomas RJS, and Burke JF: Postburn hypermetabolism: Relation to evaporative heat loss and catecholamine level. *Surg Forum* 26:74-76, 1975.
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12. Wilmore DW, Mason AD, Jr., and Pruitt BA, Jr.: Insulin response to glucose in hypermetabolic burn patients. *Ann Surg* 183:314-320, 1976.
13. Wachtel TL, Shuck JM, Schade D, Eaton PR, and Shuck LW: Hyperglucagonemia and hepatic ketogenesis in burned swine. *J Trauma* 18:248-253, 1978.

In an earlier preliminary report, we showed that adipocytes isolated from the fat of burned animals appeared to be less responsive to the lipolytic effects of epinephrine than cells isolated from control animals (14). In order to expand and confirm the preliminary study, a series was completed in which glycerol production due to lipolysis was measured in burned and control rats at specific intervals post-injury. This study reflects refinements in the technical procedures and a larger subject population.

MATERIALS AND METHODS

Male Holtzman rats weighing 450-500 grams were randomly divided into two groups. One group was anesthetized (5 mg/100 G sodium pentobarbital), shaved and subjected to a 60% total body surface burn by scalding (15). The other group was treated the same except for the injury. All animals were housed individually in a 25°C room and allowed free access to food and water.

Animals from both groups were selected randomly for sacrifice on days 1, 5, 10, 15 and 20 post-injury. After a 17-hour fast, the animals were decapitated and the epididymal fat pads removed and placed in warm Krebs-Ringer bicarbonate buffer (KRB). After trimming, the distal portions of the pads were minced and digested with collagenase (Worthington, Lot 40K043, 3 mg/ml) in KRB containing 4% albumin fraction V (Sigma, Lot 80F07071). Buffer solutions were equilibrated with 5% CO₂-95% O₂ at all times during the experiment. Adipocytes isolated by the digestion were freed from tissue matrix by filtering through a 105 nylon mesh, washed three times and suspended in a known volume of KRB-albumin.

Duplicate 5 ml aliquots of cell suspension were incubated at 37°C with gentle shaking for 60 minutes. Epinephrine was present in one pair of samples at a final concentration of 10⁻⁵M. The second pair contained no hormone and served as controls. At the conclusion of the incubation period, the samples were added to 0.5 ml cold trichloroacetic acid (TCA; 50% w/v). A third pair of samples was added to TCA immediately upon dispensing to provide pre-incubation values. All samples were filtered and the filtrates stored at -20°C until analysis.

The filtrates were analyzed for glycerol content by enzymatic spectrophotometric assay after TCA extraction with diethyl ether. The difference between glycerol content at 60 minutes and that at time zero equalled glycerol production by nmoles/ml/h. These values were normalized per 10⁶ cells by counting under a microscope the number of cells in five aliquots of cell suspension (5 µl) which had been fixed in 2% osmium tetroxide (Degussa Corp.).

14. Strome DR, Goodwin CW, and Mason AD, Jr.: The role of lipid metabolism in burn injury: I. The effect of epinephrine on adipocyte function. USAISR Annual Research Progress Report, FY 1980, 322-329.

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RESULTS

Table 1 presents glycerol production with postburn day and the increase in glycerol production due to epinephrine stimulation. Each value represents data collected from four animals. Basal rates of glycerol production were not different in the burned and unburned groups. However, the response of the adipocytes to epinephrine stimulation was depressed with respect to controls as early as the fifth day postburn in the burned animals and remained depressed for the duration of the experimental period. Control rats, on the other hand, showed a sustained level of response throughout the 20 days post-treatment. Although there appeared to be a slightly depressed response on day 1, this was not statistically important (ANOVA).

TABLE 1.
GLYCEROL PRODUCTION (NMOLES/10⁶ CELLS-H)

POSTBURN DAY		1	5	10	15	20
<u>BASAL RATES</u>						
CONTROL	\bar{X}	109	292	321	166	-47
	SE	154	109	132	86	187
BURN	\bar{X}	-42	109	75	194	58
	SE	44	102	120	88	223
<u>STIMULATED</u>						
CONTROL	\bar{X}	957	1873	1540	1478	1261
	SE	185	681	130	360	228
BURN	\bar{X}	1368	338**	576**	411**	475**
	SE	692	227	78	147	352
<u>DIFFERENCE</u>						
CONTROL	\bar{X}	848	1581	1219	1312	1308
	SE	207	586	175	397	328
BURN	\bar{X}	1409	230**	501**	217**	417**
	SE	667	209	66	89	225

**p < 0.01 burn vs control by ANOVA.

Figure 1 shows the changes in body weight observed in the two groups of animals which were taken to 20 days. In all cases, the burned animals lost body weight over the experimental course. Animals which had been sham burned lost weight on the first day after treatment and then gained weight at a rate similar to untreated animals, resulting in an apparent lack of weight change. No attempt was made to match experimental and control animals with respect to weight or food intake. This would have required force-feeding the burned animals or imposing partial starvation on the controls. Both interventions were deemed unnecessary at this level of investigation.

DISCUSSION

The role of fat in postburn hypermetabolism is still mostly undefined. It is clear that the use of fat for oxidative energy is increased following most injuries (1, 16), necessitating higher rates of mobilization of free fatty acids from adipose tissue. This elevated lipolysis is associated with, and likely linked to, the high levels of circulating catecholamines which are evidenced by enhanced excretion rates during the post-injury period (8, 10).

This postulated link between hypermetabolic lipolysis and catecholamines in burn injury has received some attention. Aprille et al (10) have shown that β -receptors in adipose tissue from burned animals do not desensitize in terms of C-AMP production with either multiple acute or chronic catecholamine exposures. They also found that adenylate cyclase activity was increased equally in burned (20%), sham-burned and normal rats upon initial exposure to isoproterenol. It should be recognized that the conditions of incubation are extremely important in measuring desensitization (17, 18) and that changes in cyclic AMP are not directly translatable to changes in lipolysis (19). However, the logical conclusion of the experiments of Aprille et al is that adipose tissue would maintain normal or elevated ability to respond to lipolytic demands by catecholamines even after prolonged exposure such as occurs in the postburn course.

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17. Grundler ML, and Bernstein RS: Segmental Lipolysis in rat tissue upon repeated exposure to epinephrine. *Metabolism* 28:989-993, 1979.

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Our findings suggest quite the opposite, that is, the ability to respond lipolytically to 10^{-5} M epinephrine was actually depressed in adipocytes from burned animals when compared to controls beginning as early as the fifth postburn day. Possible explanations for the apparent difference are burn size (60% vs 20% total body surface) and environment. It is known that hypermetabolism can be minimal in the rat at moderate burn sizes when housed in a warm room and that increased extent of burn is associated with increased hypermetabolism (20). Alternatively, it is possible that the change in the lipolytic pathways causing this response may be distal to the involvement of adenylate cyclase. In any case, the depression in hormonal responsiveness in adipocytes from burned animals shows that significant alterations have occurred at the cellular level in adipose tissue as a result of burn injury.

The position which this response occupies in the integrated metabolic reaction to burn injury is not clear from investigation in a controlled and isolated system. Concerning the hormonal situation in vivo, after one week, insulin and glucagon are back to, or near, normal (11, 12), leaving only circulating epinephrine and norepinephrine as primary lipolytic hormones. In terms of other metabolic pathways, provision of excess glucose does not appear to offset fat oxidation (1, 16) or tricycleride breakdown (4), and amino acids seem to be mainly devoted to gluconeogenic pursuits (2). One suggestion is that the rate of lipolysis in the burned animal is actually less than what would be observed if normal hormone responsiveness persisted. This would help to reduce the rate at which fat stores are depleted, prolonging substrate availability.

The immediate cause of this loss of hormone responsiveness is not known; however, there are several possibilities which will be dealt with in outline form below.

1. Collagenase treatment is known to alter adipocyte function in some cases (21). All cells were treated in the same manner. Therefore, it is unlikely that digestion alone could cause the findings, unless the cells are differentially altered by the treatment.

20. Herndon DN, Wilmore DW, and Mason AD, Jr.: Development and analysis of a small animal model simulating the human postburn hypermetabolic response. *J Surg Res* 25: 394-403, 1978.

21. Kono T, and Barnham FW: Insulin-like effects of trypsin on fat cells. Localization of the metabolic steps and cellular sites affected by the enzyme. *J Biol Chem* 246: 6204-6209, 1971.

2. Incubation of isolated adipocytes can lead to buildup of inhibitory metabolites such as adenosine (22, 23) and fatty acids (24) in the incubation medium. The number of cells per ml of medium was not different in the two groups and was kept below or at 100,000 cells per ml. This concentration has been shown to avoid such inhibitory situations in normal animals (25). To produce our results, the cells from burned animals would have to release more fatty acids or adenosine per unit time. This possibility will be investigated in the future.

3. There could be alterations in receptors (sensitivity, density) or in receptor-hormone interactions which could cause such a loss of responsiveness. Since the maintenance or loss of response to catecholamines in adipocytes is variably reported during chronic stimulation (17, 18) or cold stress (26), this also will need to be examined.

4. Changes in cellular enzyme levels are certainly suspect in this decreased lipolysis. Adenylate cyclase, phosphodiesterase, protein kinase and hormone-sensitive lipase can all be altered in level of activation or amount in ways which could theoretically result in inhibition of lipolysis.

5. There is evidence of an alternate route of lipolytic activation in adipocytes which is independent of the cyclic-AMP pathway (27, 28). What involvement this may have, if any, is unknown.

22. Sengupta K, Long KJ, and Allen DO: Growth hormone stimulation of lipolysis and cyclic-AMP levels in perfused fat cells. *J Pharmacol and Exper Therap* 217: 15-19, 1981.

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24. Burns TW, Langle PE, Terry BE, and Robinson GA: The role of free fatty acids in the regulation of lipolysis by human adipose tissue cells. *Metabolism* 27: 1755-1762, 1978.

25. Yu BP, Bertrand HA, and Masaro EJ: Nutrition-aging influence of catecholamine-promoted lipolysis. *Metabolism* 29: 438-444, 1980.

26. Barney CC, Katovich MJ, Fregly MJ, and Tyler PE. Changes in β -adrenergic responsiveness of rats during chronic cold exposure. *J Appl Physiol: Resp, Environ, Exer Physiol* 49: 923-929, 1980.

27. Wise LS, and Jungas RL: Evidence for a dual mechanism of lipolysis activation by epinephrine in rat adipose tissue. *J Biol Chem* 253: 1624-1627, 1978.

28. Kissebah AH, Tulloch BR, Vydellingum N, Hope-Gill H, Clarke P, and Fraser TR: The role of calcium in insulin action. II. Effects of insulin and procaine hydrochloride on lipolysis. *Horm Metabolic Resch* 6: 357-364, 1974.

6. Finally, it has been suggested that there are two pools of triglyceride in adipose tissue with different availabilities for degradation (29,30). If this is true and the rapidly available pool is very small, it is possible that prolonged elevation in lipolysis could lead to depletion of this pool. This would be reflected in decreased ability to degrade triglycerides during acute lipolytic demand such as in our experiments.

It is clear that several lines of investigation need to be pursued to completely understand the meaning of the altered cellular response and its cause. These inquiries will form the basis of future experiments.

PRESENTATIONS/PUBLICATIONS

None.

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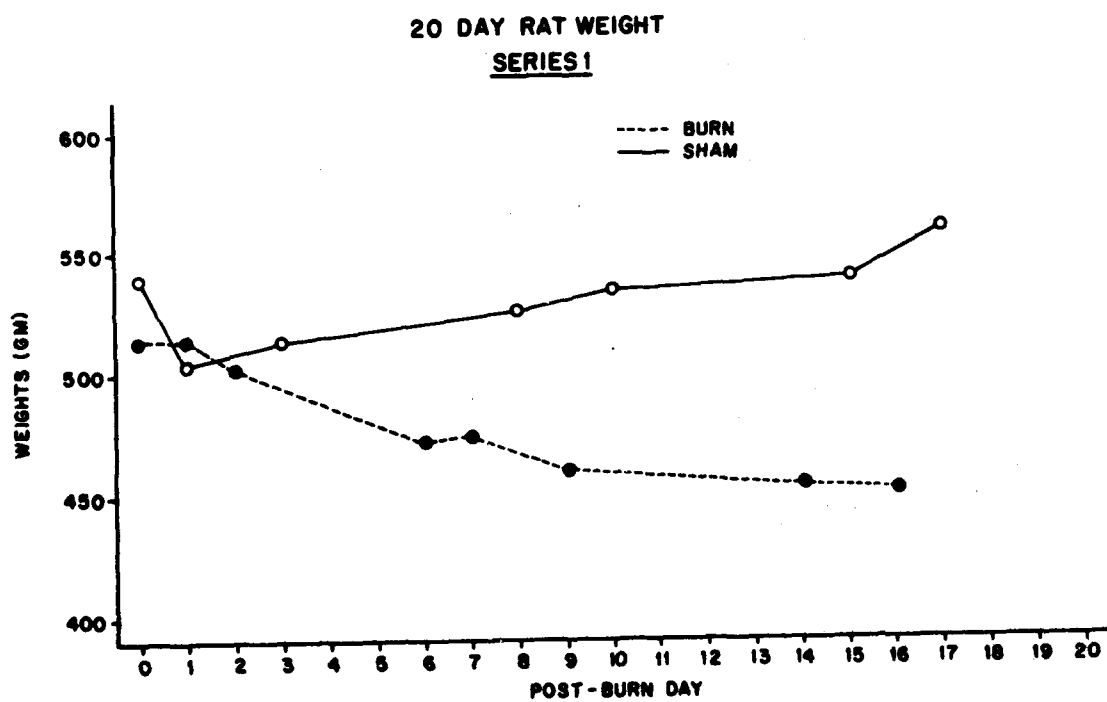


Figure 1

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DRAE(AR)636	
3. DATE PREV SUMMARY ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DES'N INSTR ^a	9. SPECIFIC DATA- CONTRACTOR ACCESS ^a	10. LEVEL OF SUM ^a
81 05 11	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
11. NO./CODES: ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		61101A		3A161101A91C		00	
b. CONTRIBUTING						088	
c. CONTRIBUTING							
12. TITLE (Precede with Security Classification Code) ^a (U) Characterization of Skeletal Muscle Metabolism After Thermal Injury (44)							
13. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine and 012900 Physiology							
14. START DATE		15. ESTIMATED COMPLETION DATE		16. FUNDING AGENCY		17. PERFORMANCE METHOD	
81 04		Cont		DA		C. In-House	
18. CONTRACT/GRANT				19. RESOURCES ESTIMATE		20. PROFESSIONAL MAN YRS	
Not Applicable				B. FREEDOMS		C. FUNDS (in thousands)	
a. DATES/EFFECTIVE:				FISCAL YEAR		1981	
b. NUMBER: ^a				CURRENCY		1.0	
c. TYPE:				1982		0.9	
d. KIND OF AWARD:				20			
e. AMOUNT:							
f. CUM. AMT.							
21. RESPONSIBLE DOD ORGANIZATION				22. PERFORMING ORGANIZATION			
NAME: ^a US Army Institute of Surgical Research				NAME: ^a US Army Institute of Surgical Research			
ADDRESS: ^a Ft Sam Houston, Texas 78234				ADDRESS: ^a Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Associate Institution)			
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: ^a James J. Newman, CPT, MSC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-2968			
23. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
24. KEYWORDS (Precede EACH with Security Classification Code) (U) Skeletal Muscle Metabolism; (U) Burn Injury; (U) Oxidative Metabolism; (U) Branched-Chain Amino Acids; (U) Laboratory Animal							
25. TECHNICAL OBJECTIVE, ^a 26. APPROACH, 27. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To evaluate the changes that occur in skeletal muscle metabolism after thermal injury and to determine means of reducing mortality due to the severe catabolic state observed in the burned soldier.							
24. (U) Using standard differential respirometry techniques, liquid scintillation counting procedures for radioassays, and enzyme concentration and kinetic measurements, the metabolic response of skeletal muscle to burn injury will be delineated.							
25. (U) 8010 - 8109. The protocol for this study has just recently been approved (20 Apr 81) and the appropriate equipment is being procured. Preliminary data on animals is being obtained to verify validity of the assay procedures to be employed.							

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

**REPORT TITLE: CHARACTERIZATION OF SKELETAL MUSCLE METABOLISM AFTER
THERMAL INJURY**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
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Basil A. Pruitt, Jr., M.D., Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

**REPORT TITLE: CHARACTERIZATION OF SKELETAL MUSCLE METABOLISM AFTER
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**US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
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Basil A. Pruitt, Jr., M.D., Colonel, MC**

Reports Control Symbol MEDDH-288(R1)

Postinjury metabolism is characterized by elevated hepatic alanine uptake and glucose production, as well as increased urea loss resulting in a negative nitrogen balance. The source of the alanine used for hepatic gluconeogenesis and of the urea nitrogen is believed to be skeletal muscle. The responses of selected regulatory enzymes, phosphofructokinase (PFK), glutamate-pyruvate transaminase (GPT), and citrate synthase (CS), involved in glycolysis, amino acid metabolism, and the citric acid cycle, respectively, were examined in rat muscle after burn injury. Male Sprague-Dawley rats with 50% total body surface scald burns were used to study the response of uninjured muscles representing a spectrum of fiber types at 3, 7, 13, and 20 days postburn. By 13 to 20 days after injury the soleus and diaphragm muscles (intermediate and "red" fiber types, respectively) showed an increased specific activity (nmoles/min/mg protein) in CS (17-22.5%), PFK (25-28%), and GPT (39-52%). The epitrochlearis (a "white" muscle) showed no change in CS activity, but PFK and GPT increased 17% and 50%, respectively, by 13 to 20 days after injury. These results indicate that muscle adapts to injury by increasing its ability to produce alanine, form pyruvate, and oxidize substrates via the citric acid cycle.

**Skeletal muscle metabolism
Burn injury
Oxidative metabolism
Branched-chain amino acids**

CHARACTERIZATION OF SKELETAL MUSCLE METABOLISM AFTER THERMAL INJURY

Burn injury causes dramatic acute and chronic physiological alterations. Burn patients exhibit a hypermetabolic period which reaches its zenith on the ninth to twelfth postburn day (1,2), and the degree of this hypermetabolic response is proportional to the extent and severity of the wound (1,3). Both injured and non-injured tissues are thought to contribute to the elevation in whole-body oxygen consumption during the hypermetabolic period (1). The elevated oxygen consumption after burn injury implies that more energy is needed by the burn patient and that more substrate must be oxidized to provide this energy. It is important to determine if this increased metabolic rate causes chronic adaptations in uninjured tissue after thermal injury.

The postburn period has been characterized by several metabolic alterations that occur after injury. The burn patient exhibits an elevated hepatic glucose production and increased hepatic alanine uptake after injury (4). Peripheral glucose utilization was shown to be unaffected until 6 to 16 days after injury, at which time the rate of glucose disappearance is elevated (5). During this period the glucose-alanine cycle proceeds at an accelerated rate (6) and the nitrogen from alanine and other amino acids is lost as urea at an increased rate, resulting in a negative nitrogen balance (4,6). Skeletal muscle is believed to be the primary source of these amino acids that are used as gluconeogenic precursors by the liver during the postburn period (4,6).

Skeletal muscle is known to adapt to changes in its homeostatic metabolic state. Exercise (7,8), selected hormonal treatment (9), and

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8. Baldwin KM, Klinkerfuss GH, Terjung RL, Molé PA, Holloszy JO: Respiratory capacity of white, red, and intermediate muscle: adaptive response to exercise. *Am J Physiol* 222:373-378, 1972.
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cold-stress (10) are all effectors of muscle metabolic adaptations. Muscle has the ability to adapt to these stimuli by selectively increasing the amount of mitochondrial protein, while other protein structures, such as the myofibrils, do not exhibit a net increase in muscle fiber protein content (7,8). This adaptation is a selective process involving an altered expression of protein synthesis. Since the unburned limb of an injured patient was shown to have elevated oxygen consumption during the postburn hypermetabolic period (1), perhaps this observation reflects a greater ability by skeletal muscle to use oxygen for cellular energy production. The purpose of this study is to determine whether muscle exhibits measurable metabolic adaptations during the postburn period. Selected regulatory enzymes involved in glycolysis, amino acid metabolism, and the citric acid cycle were examined.

MATERIALS AND METHODS

Animal care and treatment. Male Sprague-Dawley rats were used in this investigation and were maintained on a diet of Purina laboratory chow and water provided ad libitum and exposed to a 12:12-hour light-dark cycle. The animals were divided into two groups: a control group and a burned group. The animals were burned using the procedure described by Herndon et al. (11). Briefly, this procedure consists of anesthetizing the rat (50 mg pentobarbital per kg), shaving the area to be burned, placing the animal in a body mold which exposes a known percentage of the total body surface (TBS), and scalding the animal in water to produce the desired wound depth. In this experiment, the rats (180-200 g) received a 50% TBS burn (30% on the dorsum and 20% on the abdomen). In order to produce a full-thickness wound and minimize damage to underlying tissues, the dorsum was scalded for 9 seconds and the abdomen for 3 seconds in 98° C water. Saline (20 ml) was given intraperitoneally prior to scalding the abdomen to provide protection to the viscera and to aid in the resuscitation of the animal. Animals from each group were sacrificed at 3, 7, 13, and 20 days postinjury. Controls were sacrificed with burned animals on these selected postburn days, and all experiments were performed at the same time of day.

Tissue sampling and processing. Selected muscles representing a broad spectrum of fiber types were removed from anesthetized (50 mg/kg, pentobarbital) rats on the specified postburn day. The soleus, epitrochlearis, and diaphragm were chosen for study. The soleus is classified as an intermediate fiber type muscle (intermediate oxidative capacity, low glycolytic capacity), the diaphragm is a "red" muscle (high oxidative capacity, moderate glycolytic capacity), and the epitrochlearis is a "white" muscle (low oxidative capacity, high glycolytic capacity). The muscles were dissected free of connective tissue, minced on ice, and weighed prior to dilution for homogenization. A 5% (w/v) homogenate was prepared from

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each muscle with a medium consisting of: 175 mM KCl, 10 mM glutathione, and 2.0 mM EDTA (pH 7.0). Each sample remained chilled during homogenization with an Ultra-Turrax homogenizer (Tekmar Ind., Cincinnati, Ohio). A portion of the whole homogenate was centrifuged at 1,000 g for 10 minutes. The supernatant and remaining whole homogenate were stored at -80°C until analysis.

Enzyme assays. All assays were performed in 1 ml cuvettes of 1 cm light path at 30°C . Reaction rates were measured at zero-order kinetics, and the rates were proportional to the protein concentration of the samples.

Citrate synthase, CS (citrate oxaloacetate-lyase, EC 4.1.3.7), activity was assayed as described by Srere (12) with the use of 5,5'-dithiobis-(2-nitrobenzoic acid), DTNB. Final reagent concentrations used in this assay included: 60 mM Tris buffer (pH 8.0), 300 μM acetyl-CoA, 100 μM DTNB, 500 μM oxaloacetate, 3.0 mM K_2HPO_4 , and diluted homogenate equivalent to 0.1 mg wet weight of tissue.

Proospho-fructokinase, PFK (ATP: D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11), activity was measured in the 1,000 g supernatant using the assay procedure described by Bergmeyer *et al.* (13). The final reagent concentrations in the reaction mixture were: 70 mM Tris buffer (pH 8.5), 1.4 mM MgSO_4 , 4.5 mM KCl, 0.71 mM phosphoenolpyruvate, 0.64 mM fructose-1,6-diphosphate, 1.8 mM fructose-6-phosphate, 1.1 mM ATP, 0.4 mM NADH, 4.2 U/ml pyruvate kinase, 9.6 U/ml lactate dehydrogenase, and enough sample to provide 1.0 mg wet weight of tissue.

Glutamate-pyruvate transaminase, GPT (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2), activity was measured using the spectrophotometric assay described by Bergmeyer and Bernt (14). Optimum conditions included the following reagents in the assay mixture: 80 mM potassium phosphate buffer (pH 7.4), 18 mM α -ketoglutarate, 600 mM L-alanine, 0.18 mM NADH, 1.2 U/ml lactate dehydrogenase, and enough homogenate to provide 1.0 mg wet weight of tissue.

Sample protein content was determined using the biuret method described by Gornal *et al.* (15). Bovine serum albumin was used as the standard for determining protein concentrations.

Analysis of results. The analysis for statistically significant alterations in enzyme activity over the postburn period was performed using

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a one-way analysis of variance for each muscle for each enzyme. The difference in activities between muscles is well documented and was not of primary interest in this study.

RESULTS

Body weight response. Previous work from this laboratory determined that uninjured control male Sprague-Dawley rats gain 6-7 grams per day over the 180-350 g body weight range (11). The control animals in this study gained weight at 6.6 ± 0.5 g per day, but the burned group gained weight at 3.9 ± 0.4 g per day over the 20-day postburn period ($P < 0.01$).

Enzyme activities. Citrate synthase activity was measured in each muscle (Table 1). An increase in citrate synthase activity was observed in the soleus (13%) and diaphragm (17%) of burned rats at 13 days postinjury and the levels of citrate synthase remained elevated at 20 days postburn. The epitrochlearis muscle showed no significant change in citrate synthase activity over the postburn period. The protein content per gram wet weight of muscle in the injured animals was not significantly different from control values at any postburn time period, indicating that no significant edema or change in protein concentration occurred within these muscles after injury.

Phosphofructokinase (PFK) activity was assayed in each muscle at selected postburn time periods (Table 2). PFK activity in the soleus and diaphragm was significantly higher (28% and 25% respectively) by the twentieth postburn day. The epitrochlearis showed a 17% increase in PFK by the twentieth postburn day.

Glutamate-pyruvate transaminase (GPT) activity was assayed in each muscle during the postinjury period (Table 3). The GPT activity in the soleus and diaphragm maximized by the thirteenth to twentieth postburn day, with the GPT activity increasing 52% in the soleus and 39% in the diaphragm. By the thirteenth day after injury GPT activity in the epitrochlearis increased 50% above control levels.

DISCUSSION

Thermal injury causes several metabolic alterations in uninjured skeletal muscle. In this study the burned animals had a significantly slower growth rate, and smaller muscles, in comparison to uninjured control rats. These results confirm previous observations that showed injured animals or patients with unlimited access to food do not maintain their preinjury weight (11,16). These results have been interpreted (17) to mean that the burned animal does not have the ability to utilize enough

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TABLE 1. CITRATE SYNTHASE ACTIVITY IN RAT MUSCLE AFTER BURN INJURY

	SOLEUS	DIAPHRAGM (nmoles/min/mg protein)	EPITROCHLEARIS
Controls (6)	110.3 ± 7.3	187.3 ± 12.2	81.8 ± 2.3
3 days postburn (5)	113.4 ± 7.7	190.3 ± 6.1	61.0 ± 7.2
7 days postburn (5)	118.0 ± 3.4	192.0 ± 8.0	77.5 ± 5.3
13 days postburn (10)	123.6 ± 3.9	219.0 ± 8.7*	85.3 ± 4.1
20 days postburn (5)	135.2 ± 9.8*	201.8 ± 16.0	69.7 ± 6.2

Values are means ± SEM, number in () is N per group.

* P < 0.05 vs controls.

TABLE 2. PHOSPHOFRUCTOKINASE ACTIVITY IN RAT MUSCLE AFTER BURN INJURY

	SOLEUS	DIAPHRAGM (nmoles/min/mg/protein)	EPITROCHLEARIS
Controls (6)	34.3 ± 1.8	80.3 ± 4.6	149.3 ± 4.8
3 days postburn (5)	35.8 ± 1.3	80.1 ± 7.5	117.9 ± 13.7
7 days postburn (5)	30.1 ± 1.7	70.2 ± 2.4	128.4 ± 7.3
13 days postburn (10)	39.2 ± 2.8	94.8 ± 5.8*	163.5 ± 6.0
20 days postburn (5)	43.8 ± 3.0*	100.2 ± 5.6*	174.2 ± 13.3*

Values are means ± SEM, number in () is N per group.

* P < 0.05 vs controls.

TABLE 3. GLUTAMATE-PYRUVATE TRANSAMINASE ACTIVITY IN RAT MUSCLE AFTER BURN INJURY

	SOLEUS	DIAPHRAGM (nmoles/min/mg protein)	EPITROCHLEARIS
Controls (6)	20.6 ± 1.4	36.3 ± 1.2	24.4 ± 1.6
3 days postburn (5)	24.6 ± 1.7	35.1 ± 2.4	19.0 ± 2.3
7 days postburn (5)	28.1 ± 2.3	38.1 ± 3.1	30.1 ± 1.6
13 days postburn (10)	30.3 ± 1.7*	50.2 ± 3.2*	36.6 ± 2.4*
20 days postburn (5)	31.4 ± 2.6*	50.4 ± 3.4*	31.4 ± 2.7*

Values are means ± SEM, number in () is N per group.

* P < 0.05 vs controls.

substrate to produce energy and/or precursors for wound healing and anabolic metabolism simultaneously.

Selected rat muscles were examined for changes in maximal enzyme activity over the postburn period. The enzymes chosen for study were citrate synthase, glutamate-pyruvate transaminase, and phosphofructokinase. Citrate synthase is a citric acid cycle enzyme located in the mitochondrial matrix and is often used as a marker for mitochondrial content in muscle (7,8). Glutamate-pyruvate transaminase is a cytoplasmic enzyme involved in the transfer of amino groups from free intracellular amino acids to pyruvate to form alanine. Phosphofructokinase is a cytoplasmic glycolytic regulatory enzyme involved in the oxidation of glucose and/or glycogen through the glycolytic pathway.

In this investigation an elevation in the specific activity of citrate synthase was observed in the soleus and diaphragm muscles by 13 days after injury. This observation indicates that these muscles are responsive to some stimuli during the postburn period which cause a change in citrate synthase synthesis and/or degradation. If citrate synthase reflects the response of other citric acid cycle enzymes, then they too would adapt during the postinjury period, indicating that an increase in mitochondrial content occurs in these muscles. Elevated mitochondrial content increases the oxidative capacity of the muscle and, therefore, the ability of the muscle to use oxygen to produce ATP. The elevation in citrate synthase and oxidative capacity of these muscles provides an explanation for the elevated oxygen consumption observed across the uninjured limb in burn patients (1). The time frame for this adaptation to occur in burned rats is similar to the time required for the development of the hypermetabolic period in burn patients. Both the hypermetabolic period in patients and the increase in citrate synthase in rat muscle occur by 13 days after injury, but are not measurable at 7 days postburn.

Both GPT and PFK follow a time course of adaptation similar to CS in the soleus and diaphragm. GPT increases to significantly higher levels by 13 days postinjury. These results indicate that the maximal capacity for producing alanine from pyruvate increases significantly in these muscles by 13 days after injury. These results suggest that the glucose-alanine cycle is operative at a faster rate. Burn patients exhibit elevated alanine in the blood, as well as higher hepatic alanine uptake and glucose production (6). Other investigators have hypothesized that the alanine comes from skeletal muscle (4,6). These results provide circumstantial evidence that muscle does produce higher amounts of alanine as reflected by increases in GPT after burn injury.

The elevated levels of muscle PFK observed in this study after burn injury also provide further evidence that glucose and/or glycogen are being utilized at elevated rates after injury. Although glycolysis generates very little energy, the pyruvate formed from glycolysis can be oxidized in the citric acid cycle or used for transamination to form alanine. Since GPT and CS are elevated in muscle after injury, it is likely that the pyruvate is used for transamination to form alanine and oxidized to CO_2 at higher rates.

Interestingly, the epitrochlearis muscle did not show an increase in CS during the postburn period. However, GPT and PFK were elevated by 13-20 days after injury. These results indicate that the oxidative capacity of the epitrochlearis was unaltered, but the capacity for glycolysis and transamination was elevated. The epitrochlearis is classified as a "white" fiber type muscle (18). White muscles have a very low oxidative capacity normally and derive most of their energy from glycolysis. Generally, white muscles are not recruited to aid in movement unless the movement is vigorous (7,8), whereas "red" and "intermediate" muscles are employed constantly to maintain posture and perform everyday movements such as walking and eating. Furthermore, the white muscles are poorly vascularized and the red and intermediate muscles are well vascularized (8).

Considering these differences in function and anatomy of white muscle, the following hypotheses are suggested to explain the lack of adaptation by CS in the epitrochlearis:

- (1) The blood supply to an already poorly supplied white muscle may decrease or remain unchanged, thereby not altering substrate availability to the white fiber type muscles.
- (2) Since white muscles are not used to a large extent in the injured animal, this inactivity could lead to no adaptation in oxidative capacity.

These explanations are plausible in light of the work of other investigators. Aulick *et al.* (19) showed that although oxygen consumption is elevated in the unburned limb of patients, the leg blood flow was not altered. Perhaps this lack of an increase in flow is primarily observed in white skeletal muscle. If the blood flow and substrate delivery to white muscle is substantially less than delivery to red and intermediate muscle, the lack of CS adaptation in the epitrochlearis could be related to a lack of substrate availability for increased protein synthesis.

Inactivity could also be an explanation for decreased citrate synthase activity seen in the epitrochlearis. Other investigators have shown a significant decrease in skeletal muscle mitochondrial protein and oxidative capacity after only 2 days of limb immobilization (20). These results suggest that a lack of contractile activity by white muscle is counterproductive to increases in oxidative capacity.

The results of this study indicate that metabolic adaptations occur in uninjured muscles in response to some signal after burn injury. These

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19. Aulick LH, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Muscle blood flow following thermal injury. *Ann Surg* 188:778-782, 1978.

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adaptations maximize at approximately the same time as the hypermetabolic response reaches its zenith. Red and intermediate muscles are more prone to show adaptive responses in oxidative capacity, whereas white muscle does not demonstrate an increase in citrate synthase activity within the same time frame as the red and intermediate fiber type muscles. The fact that uninjured muscles show adaptations after burn injury provides evidence that some pervasive signal is causing changes in muscle metabolism regardless of the proximity to the injury site.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION#	2. DATE OF SUMMARY	REPORT CONTROL NUMBER	
				DA OG 6976	81 10 01	J.D-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCY#	6. WORK SECURITY	7. REGRADING	8. DESIGN INSTR#	9. SPECIFIC DATA - CONTRACTOR ACCESS	
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10. NO./CODES#		PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
A. PRIMARY		61101A	3A161101A91C	00	089		
B. CONTRIBUTING							
C. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) (U) Use of a Laminar Flow Isolator to Control Infection In Burned Troops (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS#							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
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17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDENCE		B. FUNDS (\$ thousands)	
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C. TYPE:				1981		0.5	
D. KIND OF AWARD:				1982		0.2	
E. CUM. AMT.				19			
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research			
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Precede with U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr., COL, MC				NAME: William F. McManus, COL, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-3301			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
23. REVISIONS (Precede with Security Classification Code)							
(U) Burn Injury; (U) Infection; (U) Laminar Flow; (U) Humans; (U) Wound Colonization							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRAM (Precede individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) It has been well known in recent years that the development of infection has been the most common cause of death in burned soldiers. As the vast majority of these cases result from invasive infection of the burn wound, methods of reducing burn wound contamination would be expected to result in improved survival. In addition, studies have shown that cross-contamination colonization causes more invasive burn wound infections than auto-contamination colonization. These facts generated interest in the use of laminar air flow isolator units as part of burn care.</p> <p>24. (U) The Sci-Med Company of Minneapolis, Minnesota, was contracted to develop a Laminar air flow unit to meet certain specifications. Following temporary installation and initial patient trials, necessary modifications were undertaken and the unit was redesigned and replaced. Comparison of burn wound colonization between laminar flow and conventionally treated patients is now in progress.</p> <p>25. (U) 8010 - 8109. Initial experience with the new laminar flow unit indicates a marked increase in nursing care requirements for each patient; visual wound inspection has become markedly more difficult; small children suffer from emotional and physical deprivation due to a</p>							

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL DD-DR&E(AR)496	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ²	6. WORK SECURITY ³	7. REGRADING ⁴	8. DESGN INSTRN	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM
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11. NO./CODES: ⁵		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
A. PRIMARY		61101A		3A161101A91C		00	
B. CONTRIBUTING						089	
C. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ⁶ (U) Use of a Laminar Flow Isolator to Control Infection In Burned Troops (44)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁷ 003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
77 09		Cont		DA		C. In-House	
17. CONTRACT/GRANT Not Applicable				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDENCE		B. FUNDS (in thousands)	
B. NUMBER: ⁸				FISCAL YEAR		1981	
C. TYPE:				CURRENT		0.5	
D. KIND OF AWARD:				1982		0.2	
E. CUM. AMT.						19	
20. RESPONSIBLE OOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: ⁹ US Army Institute of Surgical Research				NAME: ⁹ US Army Institute of Surgical Research			
ADDRESS: ⁹ Ft Sam Houston, Texas 78234				ADDRESS: ⁹ Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)			
NAME: Basil A. Pruitt, Jr., COL, MC				NAME: ⁹ William F. McManus, COL, MC			
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-3301			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
				POC: DA			
23. KEYWORDS (Precede EACH with Security Classification Code) (U) Burn Injury; (U) Infection; (U) Laminar Flow; (U) Humans; (U) Wound Colonization							
24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.) lack of physical contact; and, in one patient colonization of the burn wound with coagulase positive <u>Staphylococcus aureus</u> occurred within 48 hours following admission to the laminar flow unit. Additional experience with this unit will fully delineate the advantages and disadvantages of laminar flow isolation in burn patients.							

DD FORM 1496

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1496A, 1 NOV 66 AND 1496-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

**PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT
RESEARCH**

**REPORT TITLE: USE OF A LAMINAR FLOW ISOLATOR TO CONTROL
INFECTION IN BURNED TROOPS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1980 - 30 September 1981

Investigators:

**William F. McManus, M.D., Colonel, MC
Robert B. Lindberg, Ph.D.
Judith Fitzpatrick, Captain, ANC
Arthur D. Mason, Jr., M.D.**

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT
RESEARCH

REPORT TITLE: USE OF A LAMINAR FLOW ISOLATOR TO CONTROL
INFECTION IN BURNED TROOPS

US Army Institute of Surgical Research, Brooke Army Medical
Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1980 - 30 September
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Infection has continued to be the most common cause of morbidity and death in burned soldiers. Because of the well documented alterations of host resistance, the burn patient is especially liable to colonization and subsequent infection of the burn wound with a variety of microorganisms including bacteria, fungi and viruses. Since some studies have suggested that cross-contamination colonization may be responsible for more burn wound infection than auto colonization, the Sci-Med Company of Minneapolis, Minnesota was contracted to develop a laminar flow unit suitable for burn patient care.

Patients admitted to this Burn Center within 24 hours of injury with burns that exceed 40% of the body surface were eligible for study in the laminar flow unit. Microbiologic monitoring of the burn wound was accomplished prior to placing the patient in the unit and daily wound cultures are obtained for the 10 days. Conventional wound care and topical chemotherapy is provided within the unit. The controls are patients in adjacent beds in the Intensive Care Unit who have similar wounds and are cultured with the same frequency as the patient in the laminar flow unit.

During the period 1 October 80 through 30 September 81 the laminar flow unit was unusable for six months. In addition when the unit was accessible it could not be used because of repeated malfunctions. The two main mechanical problems with the laminar flow isolator unit were repeated water leaks and a lack of temperature control.

Three patients have been admitted to the Unit. The first patient, a 15 month girl, refused to eat since she needed to be held while being fed. Her sensory deprivation necessitated discontinuance of the trial after seven days. The second patient was a 66 year old man who was removed from the laminar flow unit after four days when his burn wounds became colonized with fungi. The third patient was a 44 year old man whose care necessitated his removal from the laminar flow unit within 24 hours to place a Swan-Gantz catheter, arterial lines, and perform repeated bronchoscopy to maintain a clear airway.

In summary, nursing care requirements are markedly increased in the laminar flow isolator. Visibility of the patient is decreased. There is increased difficulty in management of intravenous or intra-arterial cannulae and infusions. There is increased difficulty of care when ventilatory support is required. Sensory deprivation and increased difficulty in obtaining laboratory samples or in the provision of wound care markedly complicate care. Two of the three patients who were within the laminar flow isolator for four or more days were rapidly colonized (one patient with coagulase positive Staphylococcus aureus two days after admission and the second patient with fungi) despite the ability of the laminar flow unit to maintain a clean internal environment when no patient was in the unit. The increased difficulty of care and the decreased ability to monitor critically burned patients speak for the discontinuance of this protocol since no overwhelming microbiologic advantage is apparent to counterbalance the obvious shortcomings.

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